



AGRICULTURAL RESEARCH INSTITUTE  
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# ANNALS OF BOTANY

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# An Annotated List of Groups of Wild Hybrids in the New Zealand Flora.

BY

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AND

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INTRODUCTORY.

THE period 1912-32 saw a remarkable advance in the knowledge of wild hybridism in the New Zealand Flora. Cockayne in 1912 (27) referred with some doubt to five possible hybrids, but suggested that hybridism might play a larger part than had been suspected. In 1917 (28) appeared his paper on 'Species' and 'Variety'. In the light of the taxonomic views there expressed, the study of wild hybrids was taken up in earnest by that worker and his colleagues. In 1921 came Cockayne's discovery of the vast extent of hybridism in *Nothofagus*. In 1923 (30) he published a list of 130 supposed hybrids, and attempted a classification of these on geographical distribution and opportunity to cross. The visit of the late Dr. J. P. Lohse and his public lectures on 'Evolution considered in the Light of Hybridisation' lent their impetus to the movement. As a supplement to those lectures Cockayne (31) listed 208 known hybrid groups. Studies of particular groups began to appear, and in 1928 (33, p. 401) Cockayne, in a brief summary of the results, put the number of groups at 290, considering 230 of them to be beyond doubt.

An account of the chief facts, with a special reference to his own studies in *Gaultheria*, was given by Sir Arthur Hill in 1929 before the Linnean Society of London (47). A rather detailed account of the whole subject was given by Allan (13) to the Australian and New Zealand Association for the Advancement of Science. At the present stage in the history of the investigations it seems necessary to present the following list of 491 groups, with some notes on important features of these.

A discussion of the extent of wild hybridism in the New Zealand Flora is not of local interest merely. It is the story of happenings in an area long isolated, then gradually coming more and more under the influence

of man. While it shows that hybridism was prevalent in the primitive vegetation, it also shows how man's actions have in many instances favoured the increase of hybridism in various ways—removal of forest giving fresh areas for rapid successional stages of vegetation, with increased opportunity and space for species to meet and hybridize, the bringing into proximity of species that were previously more or less isolated by their occurrence in different associations, the coming in of a new flora and vegetation with opportunity for exotic species to hybridize with indigenous ones, and so on.

All this must have an important bearing on studies in long-settled lands, and should emphasize the need for critical examination of the role of wild hybrids in their floras and vegetation. Further, the list provides the opportunity of comparing the relative incidence of hybridism in families and genera in other countries. How important *opportunity* for crossing is, may be seen from the fact that both in a garden in Scotland and one in New Zealand has been observed a hybrid between an Australian and a New Zealand *Olearia*, while several hybrid plants have been discovered in New Zealand gardens between species that never meet in nature. Whatever views be held on their significance, whatever theories be based on them, the facts here adduced must be reckoned with in all branches of botanical work.

Our taxonomic terms and conceptions have been dealt with in some detail elsewhere (6, 13, 38, 39), and have been summarized by Cockayne (38, p. 6). Here it will be sufficient to repeat that we take as our fundamental field-unit the *jordanon*, defined as 'a group of externally alike individuals which breed true when bred among themselves'. Species we class as *Simple* when consisting of only one jordanon, *Compound* when of two or more. In the floras very many 'species' are aggregates of jordanons and their hybrids; such we term *linneons*. The term *Hybrid* we apply to a plant arising from a cross, whether such crossing occurs between jordanons, a jordanon and a hybrid, or between two hybrids. An outstanding result of studies in New Zealand is the establishment of the facts that in the majority of cases the hybrid progeny show great polymorphy, and that in very many cases the hybrids show a high degree of fertility, even when the cross is between morphologically extremely distinct jordanons. Instances are not wanting where characters have arisen that are not shown in the parents. A particular series of hybrids occurring in the field we call a *Hybrid Swarm*. The total of forms found in the various swarms between a particular pair of jordanons we call a *Hybrid Group*.

It is now well known that true-breeding forms may arise as a result of hybridism. Such, for us, are nevertheless jordanons, and their origin by hybridization cannot certainly be demonstrated in the field, although field observation may give pregnant suggestions as to jordanons likely to yield



evidence on this point. We recognize fully the value of exact genetic studies, but we maintain that proper field observation can establish beyond doubt many groups of hybrids. Further strong support can be, and has in some cases already been, given by studying the results of sowing seed from supposed hybrid plants. Nor must it be forgotten that much may be learnt from the examination of wild seedlings in relation to the adults present. The evidence afforded by distribution is of great moment—case after case can be demonstrated where two jordanons show perfect uniformity in those parts of their area where they grow alone, but polymorphy at once occurs where the two meet. Those cases that have been tested by artificial hybridization have given full support to views based on field studies. We recognize fully the need for great care in attributing hybridism to aberrant forms and for scrupulous consideration of the evidence. Especially must the phenomena of epharmony be carefully examined. We use the term *Epharmonie* to apply to those forms that differ from the 'normal'—or rather, 'usual'—owing to the influence of some special environmental condition. Several examples may be found in the New Zealand literature, where such unstable epharmones have been given at least varietal rank.

We have in several publications used for hybrid groups names compounded of the specific names of the parents, e.g. the group produced by *Hebe elliptica*  $\times$  *salicifolia* we have called  $\times H. ellipsala$ . This method is not altogether original with us! De Candolle, in his 'Physiologie Végétale' of 1832, gives several examples from Schiede, and others could be cited. But the practice has met with some criticism, although the compounding of generic names in inter-generic crosses is a recognized custom. Thus Renner (59, p. 3) says, 'Es ist zu hoffen, dass dieser Brauch sich ausserhalb der Neuen Welt nicht einbürgert'. He refers, however, specifically to some of our first, rather crude, efforts at providing very brief compound names. Du Rietz (44) says, 'the method . . . is certainly very handy in verbal discussion, notebooks, &c., but for botanists less familiar with the flora concerned it may often be hard to understand that *Nothofagus cliffusca* means *Nothofagus cliffortioides*  $\times$  *fuscus*. . . In scientific literature I therefore prefer writing out the whole formula.' A more fundamental objection, one involved in giving a name to any hybrid group, is that such a name may be mistakenly applied to a few forms, or even a single form of that group. We so strongly wish to emphasize the distinction between a hybrid group and a single hybrid that we have in this paper used the formula only, except in those cases where a name has already been published in a valid manner, with application to the whole group. The horticulturist needs names for definite hybrid forms that are of horticultural value—such a name is  $\times$  *Rubus Barkeri*, unexceptional because every plant in existence has been derived vegetatively from the single wild plant found. Did  $\times$  *Hebe*

*ellipsala*, however, come to be used in horticultural literature one would soon find that, as the group *elliptica*  $\times$  *salicifolia* contains a number of distinct forms of garden value, the *Hebe ellipsala* of one garden was a very different thing from the *H. ellipsala* of another. That this is a very real source of confusion readers of gardening journals will know. But when Du Rietz (44) says 'Cockayne and Allan severely criticize the method of giving binary names of the ordinary type to hybrids' he misses the distinction between a hybrid and a hybrid group. Our expressed criticism was against using names that were originally applied as *specific* ones to a small part of a group, to apply to the whole hybrid group. Thus we consider it extremely misleading, and wrong in principle, to apply the name *Fagus apiculata* Colenso (the type specimens apparently all taken from one tree) to the whole polymorphic group formed by *N. Solandri*  $\times$  *truncata*.

Certain general features resulting from a consideration of lists are presented in the summary. Here we have great pleasure in acknowledging the Royal Society Research Grant that enabled us to gather from a wide area, in a comparatively short time, a store of information that otherwise would have taken years to accumulate.

## I. PTERIDOPHYTA.

Field (45) suggested that hybridism might be the key to some of the difficulties encountered by pteridologists in classifying their specimens. Until quite recently no serious work had been done to test this suggestion. The late Mr. Carse, at the suggestion of Cockayne, studied the ferns of the North Auckland Peninsula from this point of view, and concluded that hybridism was rife in *Asplenium*, *Polystichum*, and *Pteris*. Mrs. M. M. Martin has commenced a detailed study of the question—a study that has, especially in the genus *Asplenium*, already brought forth remarkable results. Over 150 hybrids have been added to the Wellington Open Air Museum.

## HYMENOPHYLLACEAE.

The New Zealand species of this family are very plastic and epharmonie readily, especially in regard to the development of hairs. This is well brought out in the papers of Holloway (48, 49). It is no easy matter to secure definite evidence in particular cases as to how far the diversity observed is due to epharmony, and how far to hybridism. We give a tentative list to indicate groups that should reward intensive study.

1. *Hymenophyllum demissum*  $\times$  *scabrum*. D. (D = Doubtful).

This probable hybrid group was first suggested by Field (45). Specimens collected by Allan and Zotov also suggest hybridism.

2. *H. flabellatum* × *rufescens*. D.

*H. flabellatum* has a wide range, *H. rufescens* is more local, and of restricted altitudinal range, but the two occasionally occur together, and intermediate forms have often been collected.

3. *H. peltatum* × *tunbridgense*. D.

Both are compound species, occupying different altitudinal belts. Intermediate forms are often met with on the line at which the lowland *tunbridgense* gives way to the montane *peltatum*. Lotsy, seeing Holloway's collection, was fairly certain that hybrids were present, and Holloway has expressed a similar opinion in a letter to Cockayne.

4. *H. sanguinolentum* × *villosum*. D.

Preliminary field studies suggest that both epharmony and hybridism play a part in the polymorphism observed.

## CYATHEACEAE.

No hybrid groups have been recorded in this family in New Zealand, but certain aberrant forms found suggest the desirability of studying *Cyathea dealbata* and *Hemitelia Smithii* as possible parents of hybrid forms. It is by no means established, also, that *C. Cunninghamii* is a 'good' species, and not of hybrid origin.

## POLYPODIACEAE.

Hybridity has been quite definitely established in this family, and the list given below is bound to be augmented before very long.

5. *Adiantum affine* × *fulvum*. D.

Cheeseman (25, p. 74) writes of *A. fulvum*, 'very close indeed to *A. affine*, with which it certainly seems to me to be connected by intermediate forms.' Our knowledge of polymorphism in the two species is not sufficient for us to declare as to hybridity or not, but the presence of connecting intermediates is so frequently due to hybridism that we have little doubt the same will prove true in this case.

*Asplenium bulbiferum*.

*A. bulbiferum* as described by Cheeseman (25, p. 50) is a linneon containing a vast number of forms, the jordanons of which have not yet been properly worked out. The late Mr. Carse, who had devoted considerable attention to the northern forms, concluded that there were four interhybridizing jordanons. It is certain that *A. bulbiferum* (*sens. ampl.*) hybridizes freely with many species, and Mrs. Martin has already established a number of these groups. Many hybrids are recognizable by possessing the bulbil-bearing character of the *bulbiferum* parent.

6. *A. bulbiferum* × *Colensoi*.

Certain forms have been illustrated by Allan (9). Mrs. M. M. Martin

has discovered a long series of intermediate forms, some of which bear bulbils. Her work is a fine example of what careful field observations can reveal.

7. *A. bulbiferum* × *falcatum*.

From an examination of many individuals growing in the neighbourhood of the common forms of *A. bulbiferum* and *A. falcatum*, Mrs. Martin considers that there is no doubt concerning this group.

8. *A. bulbiferum* × *flaccidum*.

This cross is revealed again and again throughout most of the region by highly polymorphic swarms. The dominance of the bulbil character is most marked.

9. *A. bulbiferum* × *Hookerianum*.

In certain forests the easily recognizable *A. Hookerianum*, with its small, rounded, stalked pinnules is invariable, except for epharmonic changes. But where it grows in company with the entirely different, much larger *A. bulbiferum*, large swarms of intermediate hybrids occur, and this is the case throughout the entire wide range of both species. As in the *A. Colensoi* cross, so here, one hybrid frond may be virtually pure *bulbiferum* and another on the same plant pure *Hookerianum*.

10. *A. bulbiferum* × *lucidum*.

The two species differ greatly, simple contrasting characters being the dull, deeply cut, soft, bulbil-bearing leaves in the one, and the shining, almost entire, stiffer leaves in the other. Forms closely resembling *lucidum*, but with bulbils, occur, so too almost pure *bulbiferum* leaves with the shining surface of *lucidum*. Also, leaves on the same hybrid plant may greatly differ among themselves.

11. *A. bulbiferum* × *obtusatum*. D.

Whether the fleshy-leaved rather small fern we here call *bulbiferum* should be included in that species or not, intermediates between it and *A. obtusatum* are common enough. See also remarks under No. 19.

12. *A. bulbiferum* × *Richardi*.

*A. Richardi* is found especially on shady rocks in the montane and the subalpine belts of the dry botanical districts lying to the east of the main divide of the Southern Alps, while *A. bulbiferum* is chiefly a lowland forest species. Nevertheless, the two occasionally meet and unmistakable hybrids have been met with.

13. *A. Colensoi* × *flaccidum*.

Hybrids of this group are common, and combine the long fleshy rachis of *flaccidum* with the finely cut segments of *Colensoi*.

14. *A. Colensoi* × *Hookerianum*.

Hybrids occur in abundance when these two distinct species grow

together. When *A. bulbiferum* is also present tri-hybrids almost certainly result.

15. *A. falcatum* × *flaccidum*. D.

Mrs. Martin informs us that apparent hybrids between the two species are not common, but she has seen certain different intermediate forms. One, showing the leaf-segments and fleshy texture of *flaccidum* coupled with the shining surface of *falcatum*, is in cultivation in the Wellington Open Air Museum.

16. *A. falcatum* × *lucidum*.

Mrs. Martin has collected many distinct forms of this cross in which the characters of both parents are plainly visible.

*A. flaccidum*.

The causes of the remarkable polymorphy in this great linneon are very imperfectly known. Cheeseman (25, p. 52) regards what we call epharmony as playing a large part, but there has been no critical study made. Can the epiphyte, with its pendulous fronds up to a metre or more long, really be an epharmonie of the short-leaved, erect, tufted ground forms?

17. *A. flaccidum* × *Hookerianum*.

Mrs. Martin finds this group to be rare, but the hybrids readily distinguishable. Some have a particularly dark green colour.

18. *A. flaccidum* × *lucidum*.

Hybrids of this group are not common, but are amply distinct.

19. *A. flaccidum* × *obtusatum*. D.

On sea-cliffs where the two species grow side by side intermediate forms, epharmonically thick, are common. But the form called *flaccidum* may possibly be epharmonic *bulbiferum*. These coastal ferns are much in need of experimental study.

20. *A. flaccidum* × *Richardi*.

The two have similar habitat requirements, and hybrids are common.

21. *Blechnum lanceolatum* × *membranaceum*.

The two species are very similar in general appearance, and the hybrids show no great diversity.

22. *B. lanceolatum* × *norfolkianum*. D.

Carse collected specimens to which he attributed this origin.

23. *Cheilanthes Sieberi* × *tenuifolia*. D.

Cheeseman (25, p. 68) speaks of intermediates between the two species.

24. *C. Sieberi* × *Notholaena distans*. D.

The two sometimes grow together, and both Carse and Allan have observed apparent hybrids.

25. *Doodia caudata* × *media*.    D.

Cheeseman (25, p. 63) speaks of forms of *caudata* that 'approach very close to' *media*. Carse had little doubt that the two hybridize.

26. *Dryopteris decomposita* × *glabella*.    D.

This was suggested by Field (43), and certainly forms difficult to place in either species are not uncommon.

27. *Hypolepis distans* × *rugosula*.    D.

This, and the following *Hypolepis* crosses, are given on the authority of Carse (22), who had made a special study of the genus.

28. *H. punctata* × *rugosula*.    D.

29. *H. punctata* × *tenuifolia*.

Hybrids of this origin appear to be on the increase where light is being let into forest in which the parents occur.

30. *H. rugosula* × *tenuifolia*.    D.

31. *Lindsaya cuneata* × *Lessonii*.    D.

Carse considered this group certain, but further study is desirable.

32. *L. cuneata* × (?) *microphylla*.    D.

We give this on the authority of Carse; the fern doubtfully referred by him to *L. microphylla* is common in the north-eastern part of South Island.

33. *Pellaea falcata* × *rotundifolia*.    D.

*P. falcata* is rare, and is known definitely only from a few localities. Intermediates occur, as in North Auckland, where the two species meet.

34. *Polystichum cystostegia* × *vestitum*.    D.

*P. cystostegia* is a summer-green fern of the subalpine belt, very distinct in its life-form from the forest-loving *P. vestitum*. The latter species sometimes reaches to the *cystostegia* belt in scrub-clothed gullies. Allan has collected at the heads of such gullies on Mount Peel (Canterbury) forms quite intermediate in characters.

35. *P. Richardi* × *syvaticum*.    D.

This has been suggested to us by Carse.

36. *P. Richardi* × *vestitum*.    D.

Also suggested by Carse. *P. Richardi* is certainly a compound species; it is also epharmonically active. As it occupies different habitats from those of *P. vestitum* it should not be difficult to test the presence of hybrids where the two meet.

37. *P. syvaticum* × *vestitum*.    D.

Carse had little doubt of this cross. We confess that we have inadequate field knowledge of *P. syvaticum*, and hesitate to list this group as established.

*Pteris*.

The genus is badly in need of revision. Carse, who had given a good deal of study to it, viewed the three species recognized by Cheeseman (25, p. 74) as linneons each composed of several jordanons, any of which appeared to hybridize freely when meeting with another. Our own observations are in accord with Carse's.

38. *Pteris comans* × *macilenta*.

39. *P. macilenta* × *truncula*.

## OSMUNDACEAE.

40. *Leptopteris hymenophylloides* × *superba*.

The two species frequently meet in North Island, and hybrids are common.

## OPHIOGLOSSACEAE.

41. *Botrychium australe* × *dissectum*. D.

A whole series of forms linking the *australe* with the *dissectum* extreme sometimes occur together. But in the absence of *dissectum* a considerable diversity of form is still often shown by *australe*. It remains uncertain what are the jordanons comprising the units of the series.

## II. GYMNOSPERMAE.

## PODOCARPACEAE.

Cockayne (35) gives a discussion of several supposed *Podocarpus* crosses, but no detailed investigations have yet been made in the family. The species are heteroblastic, a fact to be reckoned with in field studies. Certain possibly hybrid forms have been brought into cultivation as specimen plants, and have received *nomina nuda*.

42. *Dacrydium Bidwillii* × *biforme*. D.

The two species differ considerably in habit, but in adult foliage are easily confused, so that very careful study is required to demonstrate hybridism.

43. *D. Bidwillii* × *laxifolium*.

*D. Bidwillii* is an erect densely-branched shrub. *D. laxifolium* is prostrate, with slender trailing branches. The hybrid swarms show striking combinations of these life forms.

44. *D. biforme* × *laxifolium*. D.

45. *Phyllocladus glaucus* × *trichomanoides*. D.

Misses L. M. Cranwell and L. B. Moore have collected forms suggesting that this group occurs on the Waitakerei Ranges (North Auckland).

46. *Podocarpus acutifolius* × *Hallii*. D.

*P. acutifolius* was described by Kirk (53) as a low-growing shrub. In the locality whence the type was collected this almost prostrate form is common, but specimens also occur, with leaf and floral characters identical, as erect trees up to 12 m. high. Whether these different life forms represent different jordanons is not known with certainty, but where either occurs in company with *P. Hallii* intermediates that cannot be attributed to epharmomy also occur. *P. Hallii* × *nivalis* produces some forms that mimic the bushy form of *P. acutifolius* rather closely.

47. *P. acutifolius* × *nivalis*.

It is the occurrence of these hybrids, so easily confused with what would be expected from *P. acutifolius* × *Hallii*, that render this latter group doubtful, especially as all three species sometimes grow in company.

48. *P. ferrugineus* × *totara*. D.

A few specimens only have been seen, suggestive of this cross. So, too, for *P. spicatus* × *totara*. We refer to them owing to their importance for forestry.

49. *P. Hallii* × *nivalis*.

*P. Hallii* (a tall tree) frequently reaches the upper limits of forest, and there may meet the subalpine fell-field *P. nivalis* (a prostrate shrub). Hybrid swarms showing a range of life forms linking the two species have been met with in several localities. *P. nivalis* var. *erecta* Ckn. was described from a plant possibly belonging to this group, though it may have been an epharmome of *P. Hallii*.

50. *P. Hallii* × *totara*.

Although this group appears to be common, both species epharmome greatly when exposed to strong wind, and great care has to be observed in interpreting the diversity found in the field. Cockayne (35) has applied the name ×*P. Loderi* to this group, in honour of the distinguished horticulturist Mr. G. W. E. Loder, in whose garden in Sussex are many fine examples of New Zealand plants.

## CUPRESSACEAE.

51. *Libocedrus Bidwilli* × *plumosa*. D.

The two meet in the north of South Island, whence intermediate forms suggestive of hybridism have been obtained.

## III. ANGIOSPERMAE.

## I. MONOCOTYLEDONES.

## GRAMINEAE.

A revision of the family by Allan and Zotov, now in progress, is revealing that the species are much more polymorphic than has hitherto



been suspected. When the jordanons concerned have been more fully investigated it is probable that this list of hybrids will be greatly extended.

52. *Agrostis Dyeri* × *tenuis*. D.

*A. Dyeri* (allied to the northern *A. canina* L.) is the most widespread and abundant of the New Zealand species, occurring in one form or another in most montane and subalpine grasslands. *A. tenuis*, in several forms, is widely naturalized in the same grasslands. Intermediate forms are not infrequently met with, and studies of the leaf anatomy by Zotov support the view that the species hybridize rather freely.

53. *Arundo conspicua* × *fulvida*.

*A. fulvida* was accurately described by Buchanan (19). It hybridizes with *A. conspicua* in several localities, and the description of Cheeseman (25, p. 181) includes some of these hybrid forms.

54. *Danthonia Buchanani* × *semiannularis*.

We use the name *Buchanani* for the compound species so treated by local botanists, though it is not *D. Buchanani* Hook. f. as originally described.

55. *D. crassiuscula* × *flavescens*.

The distinct life forms of the parents make the hybrids easy to recognize. *D. flavescens* is wide-ranging, *D. crassiuscula* is confined to high altitudes.

56. *D. Cunninghamii* × *Raoulii*.

Both are compound species, and swarms between different jordanons of each have been met with.

57. *D. flavescens* × *Raoulii*.

Within the conception of both species there is extraordinary polymorphism, and hybridism occurs to such an extent that it will be a difficult task to sort out the jordanons concerned.

58. *D. nuda* × *semiannularis*. D.

The doubt here is whether the name *nuda* should be applied to the one parent. Hooker's original description of *D. nuda* does not altogether match his specimens, some of which appear to be hybrids. But there is a jordanon corresponding to his description that has been observed in the localities whence the supposed hybrids have been collected.

59. *D. pilosa* × *semiannularis*.

Both are exceedingly compound species. Jordanons of each frequently meet, and hybrid swarms are plentiful.

60. *D. semiannularis* × *setifolia*.

61. *Deschampsia Chapmani* × *tenella*. D.

This is based on herbarium evidence, and cannot be treated as certain.

The whole genus is very imperfectly known, and accurate data concerning the field occurrences of the form are lacking.

62. *Deyeuxia avenoides* × *quadriseta*. D.

The extreme forms of these species are very distinct, but numerous intermediates have been collected. Further field study is required to determine how far these forms represent jordanons and how far hybrids.

63. *D. Billardieri* × *Forsteri*. D.

*D. Billardieri* is mainly a sand-dune species. A form of the polymorphic *D. Forsteri* is common in some localities as a sand-hollow plant. Allan has collected apparent hybrids in company of the parents near Foxton, North Island.

64. *D. Forsteri* × *pilosa*.

*D. Forsteri* is a huge linneon, the forms of which have a wide range of habitats. *D. pilosa* is closely allied, and is treated by some as a variety. The two certainly hybridize.

65. *Dichelachne crinita* × *sciurea*.

Hybrid swarms between these two distinct, but compound, species have been noted in several localities.

66. *Festuca novae-zelandiae* × *Briquetii*. D.

The name *F. novae-zelandiae* is applied to a very complex group of jordanons and hybrids that has not yet been satisfactorily analyzed. *F. Briquetii* is a distinct species, but intermediates have been observed. Allan and Zotov have also collected forms that suggested hybridism between *F. novae-zelandiae* and the introduced *F. rubra*, now widely spread in several varieties.

67. *Hierochloa Fraseri* × *redolens*.

Having very similar habitat requirements these two distinct species frequently meet, and produce rather polymorphic groups of hybrids.

*Koeleria*.

Herbarium material of the forms of this genus in New Zealand shows remarkable diversity. Domin (43, p. 113) described three species and several varieties. It would appear that there are several inter-hybridizing jordanons, but the present field knowledge is quite insufficient to attempt any analysis.

68. *Microlaena avenacea* × *stipoides*. D.

*M. avenacea* is a forest species, while *M. stipoides* occurs mainly in grassland. The two sometimes meet on forest margins, and suspected hybrid forms have been met with. The status of *M. Carsei*, known only from a single locality, and of intermediate form, requires investigation.

69. *Poa anceps* × *caespitosa*.

The two species are of very distinct life forms, and hybrids have been observed in several localities.

70. *P. anceps* × *seticulmis*. D.

The coastal plant taken by Petrie as the type of his *P. seticulmis* is very different from *P. anceps*. Cheeseman (25, p. 193) says 'there is a widely spread inland state . . . which gradually varies into small and slender states of *P. anceps*.'

71. *P. caespitosa* × *Colensoi*.

The hybrids mimic *P. intermedia* very closely.

72. *P. Colensoi* × *intermedia*.

The two are so closely allied that it is perhaps preferable to treat them as varieties of a compound species.

73. *P. Kirkii* × *MacKayi*.

Polymorphic hybrid swarms have been noted in several localities.

74. *Trisetum antarcticum* × *Youngii*.

The exact limits of the compound species involved have not been worked out.

## CYPERACEAE.

This family provides perhaps the most difficult taxonomic task in the flora—the analysis of the genus *Uncinia*. This will be clear to any one who has attempted to name specimens from the keys provided by Cheeseman (25, p. 243) and Kükenthal (55, p. 57). Our treatment is only a first approximation, but it suffices to show that only in the light of hybridization and after very full field study can the genus be brought into order. Hybridism is rife in *Carex* also, and probably the situation is more complex than our list shows.

75. *Carex appressa* × *secta*. D.

*C. appressa* is very local, but sometimes grows in company with *C. secta*, and intermediates have been collected.

76. *C. comans* × *pulchella*.

The two species are closely related.

77. *C. dissita* × *Lambertiana*.

Though related the species are quite distinct and the hybrids easily recognizable.

78. *C. dissita* × *Solandri*.79. *C. Gaudichaudiana* × *subdola*.80. *C. Gaudichaudiana* × *ternaria*.81. *C. inversa* × *resectans*. D.

The two are very closely allied, and might better be treated as forming a compound species.

82. *C. Kirkii* × *trachycarpa*. D.

The two grow together on the Mount Arthur Plateau, South Island, whence Cheeseman collected the varieties *membranacea* and *elatio* of *C. Kirkii*. Herbarium evidence suggests that a hybrid swarm occurs there. It is probable, also, that *C. Kirkii* hybridizes with other species in the southern part of its range, to produce part of the 'exceeding' variability spoken of by Cheeseman (25, p. 256).

83. *C. Lambertiana* × *Solandri*.84. *C. lucida* × *testacea*.85. *C. Petriei* × *wakatipu*.86. *C. subdola* × *ternaria*.87. *C. secta* × *virgata*.88. *Eleocharis acuta* × *sphacelata*. D.

The two species differ widely, but Allan has observed intermediate forms in one locality where the two grow in company.

89. *Schoenus concinnus* × *nitens*.90. *Scirpus antarcticus* × *aucklandicus*.

Part of the difficulty referred to by Cheeseman (25, p. 221) in distinguishing among *S. antarcticus*, *S. aucklandicus* and *S. cernuus*, is certainly due to the fact that the species hybridize together.

91. *S. aucklandicus* × *cernuus*.92. *S. inundatus* × *sulcatus*.

While the species are amply distinct and are often met with in the pure state, they hybridize very readily. Cheeseman's var. *major* of *S. inundatus* may belong to the hybrid series.

93. *S. prolifer* × *sulcatus*.

*S. prolifer* is more local than *S. sulcatus*, but where they meet a polymorphic series of hybrids usually occurs. See also Carse (23).

*Uncinia*.

Cheeseman (25, p. 242) recognizes 14 species, with 14 described varieties. Kükenthal (55, p. 57) gives 13 with 18 varieties. Neither refers to hybridism as a possible explanation of the bewildering polymorphy. The hybrid groups here suggested for the most part represent intermediate connecting links, which if not considered as hybrids would make the whole of the genus one huge linneon quite useless in the study of vegetation. Cockayne has been able to study the genus in the light of Kükenthal's determinations of the forms in his herbarium, now located in the herbarium of the Dominion Museum. He also had lengthy communications with Kükenthal on the subject.

94. *Uncinia Banksii* × *filiformis*.

95. *U. Banksii* × *riparia*.

96. *U. caespitosa* × *filiformis*.

97. *U. caespitosa* × *purpurata*.

98. *U. caespitosa* × *riparia*.

99. *U. caespitosa* × *rupestris*.

100. *U. compacta* × *divaricata*.

It is a moot point whether these two, and certain unnamed jordanons, should not be dealt with as one compound species.

101. *U. ferruginea* × *uncinata*.

It is customary to unite these jordanons, or collection of jordanons, into a compound species, but the two are so distinct that they are more readily recognized in the field than almost any other species.

102. *U. filiformis* × *uncinata*.

103. *U. riparia* × *rubra*.

104. *U. riparia* × *uncinata*.

#### JUNCACEAE.

Unpublished studies by Mr. V. D. Zotov, of the New Zealand Plant Research Station, give very strong support to the list of *Juncus* hybrids here given, which is based in large part on his work. Cheeseman omits *J. effusus* from the second edition of his *Manual*, and its occurrences certainly suggest that it is an introduced species only. It has not been observed to hybridize with any of the indigenous species.

105. *Juncus acutus* × *maritimus* var. *australiensis*.

*J. acutus* is abundantly naturalized in salt-meadows near Foxton, North Island, and forms a polymorphic swarm of hybrids with the indigenous var. of *J. maritimus*. Both species belong to Buchenau's sub-genus *Junci thalassii*.

106. *J. caespiticius* × *planifolius*. D.

Mr. A. D. Beddie, as well as Zotov, has collected forms near Wellington that appear to be due to hybridism.

107. *J. luxurians* × *polyanthemus*.

*J. luxurians* Col. is given as a synonym of *J. polyanthemus* by Cheeseman (25, p. 295), but it is a very distinct jordanon superficially resembling *J. effusus*. Hybrids have been collected in several localities in North Island.

108. *J. novae-zelandiae* × *pusilla*.

The two species are very closely allied, and should perhaps be treated as jordanons of a compound species.

109. *J. pallidus* × *polyanthemos*.

110. *J. pallidus* × *vaginatus*.

111. *J. pauciflorus* × *polyanthemos*. D.

It is certainly easy to collect intermediate life forms, but both species are compound, and the existing diversity requires further study.

112. *J. polyanthemos* × *vaginatus*.

*Luzula*.

Cheeseman (25, p. 301) says, 'The Australian and New Zealand species are all very near to the protean *L. campestris*, and are so highly variable as to present an almost inextricable series of closely allied forms'. He follows Buchenau in grouping the forms attributed to *L. campestris* under seven varietal names, but remarks 'intermediates between all of them are plentiful'. Although we are far from any complete understanding of the New Zealand species, field work has already shown that many forms are quite constant when growing alone, and are so distinct that they should be given the rank of species.

113. *Luzula australasica* × *crinita*.

114. *L. australasica* × *floribunda*.

115. *L. australasica* × *migrata*.

116. *L. australasica* × *picta*.

117. *L. Cheesemanii* × *Colensoi*.

*L. Cheesemanii* occurs in company with *L. Colensoi* and *L. pumila* on the Saint Arnaud and other mountains in the north of South Island. The three species hybridize freely in any combination. The special characters of *L. Cheesemanii* make its influence readily traceable in these swarms.

118. *L. Cheesemanii* × *pumila*.

119. *L. Colensoi* × *pumila*.

120. *L. crinita* × *floribunda*.

121. *L. crinita* × *migrata*.

*L. micrantha*.

The species is very ill-defined. It appears probable that a series of inter-hybridizing jordanons occurs similar to that mentioned under No. 117.

122. *L. picta* × *ulophylla*.

This cross is recognized by Buchenau (20, p. 76), who, however, treats *L. ulophylla* as a var. of *L. racemosa*, from which it is very distinct, and which occupies a very different habitat. *L. ulophylla* almost certainly hybridizes with other species of the 'campestris' linneon.

## LILIACEAE.

123. *Astelia Cockaynei* × *nervosa* var. *sylvestris*.

Swarms of this origin are common in numerous localities at the junction of the subalpine and montane belts, where the forest-loving *sylvestris* meets the very distinct *Cockaynei*. *A. Cockaynei* apparently consists of a good many geographical jordanons, and some of these may ultimately be dealt with as separate species. Epharmony also plays no small part in the polymorphy of *Astelia* generally, thus adding to the difficulty of fixing the hybrid groups.

124. *A. Cockaynei* × *Petriei*.125. *A. Cunninghamii* × *Solandri*.126. *A. linearis* × *Petriei*. D.

*A. linearis* has a wide range. Forms suggestive of hybridism with *A. Petriei* have been met with in the Southern Alps at about lat. 45°, where both species are common.

127. *A. nivicola* × *Petriei*.

*A. Petriei*, where it occurs alone, is a well-marked jordanon, with characters separating it at once from its nearest ally *A. Cockaynei*. In the central and southern parts of the Southern Alps it meets occasionally the much smaller *A. nivicola*, and well-marked hybrid swarms occur.

128. *Cordyline australis* × *Banksii*.

While undoubtedly hybrids between these species have been observed it is possible that hybrid swarms are more frequent than is supposed. Records of unusually large *C. Banksii* may be based on hybrid forms. Seed from a cultivated plant in the Sanatorium grounds, Rotorua, sown by Cockayne, produced a polymorphic progeny.

129. *C. australis* × *indivisa*.

A few examples of this group were observed by Allan on Mount Egmont, North Island. Certain hybrid forms are also in cultivation.

130. *C. australis* × *pumilio*.

Several interesting and distinct forms of this group are known. The parents are strikingly different—*C. australis* is a large tuft-tree, *C. pumilio* is a dwarfed, almost stemless plant. One form has been named × *C. Matthewsii* by Carse (21). To apply this name to the whole group would be mischievous, especially as hybrids of this kind may come into cultivation and a distinct horticultural name would be needed for each distinct form.

131. *C. Banksii* × *pumilio*.

One form of the group has been named × *C. Gibbingsae* by Carse (22).

132. *Phormium Colensoi* × *tenax*.

Both are linneons of many forms. Where the two meet they usually produce a wide range of hybrid forms, easily recognizable owing to the very distinct forms of the capsules of the parents—erect and blunt in *tenax*; long, twisted, and drooping in *Colensoi*. The two have been artificially hybridized by Allan and Zotov.

## ORCHIDACEAE.

Probably intensive study would considerably increase the number of hybrid groups here recorded. The limited period in any one season for which many of the species are in good condition for examination adds to the difficulties of field study.

133. *Corysanthes micrantha* × *rotundifolia*.134. *Cyrtostylis oblonga* × *rotundifolia*.

Cheeseman (25, p. 356), who treats *rotundifolia* as a variety of *C. oblonga*, remarks, 'in several localities I have observed the two forms growing intermixed and gradually passing into each other'.

135. *Earina autumnalis* × *mucronata*.

Hybrids between these two species are not uncommon. Possibly *E. aestivalis* is one form of this group. Though the flowering periods are given by Cheeseman (25, p. 335) as distinct, they certainly overlap in many places.

136. *Pterostylis australis* × *Banksii*.

Cheeseman (25, p. 350) refers to the fact that the three species, *australis*, *Banksii*, and *graminea*, are linked together by intermediate forms, and field study has made it clear that this linkage is due to hybridism.

137. *P. australis* × *graminea*.138. *P. Banksii* × *graminea*.*Thelymitra longifolia*.

*T. longifolia* as at present understood is a linneon, some of the jordanons of which have flowers of different colours. Probably the group contains several jordanons that should be given specific rank.

2. *DICOTYLEDONES*.

## FAGACEAE.

For full discussion of the astonishing extent of hybridism in *Nothofagus* see Cockayne (32) and Cockayne and Atkinson (40), where the leaves of more than 100 hybrids are illustrated.

139. *Nothofagus cliffortioides* × *fusca*.

The group is polymorphic in the extreme, and some hybrids bear



leaf-characters absent in the parents. In some localities vast swarms occur, indeed, the hybrids present may outnumber the parental individuals. *N. Blairii* (Kirk) Ckn. is a name applied to a very small portion of the group.

140. *N. cliffortioides* × *Solandri*.

The two species greatly resemble each other, so that it is not always easy to determine whether any particular individual is a hybrid or not. When, however, the two species grow in proximity there is an evident polymorphy, absent where only one of the species is present.

141. *N. cliffortioides* × *truncata*.

The two species usually occupy different altitudinal belts, and hybrid swarms are local in distribution. Owing to the great resemblance between the leaves of *N. fusca* and *N. truncata*, it is not easy to determine the parentage of herbarium specimens lacking definite details as to the species present.

142. *N. fusca* × *Solandri*.

As in many parts of the region the vertical distribution of the parents differs greatly, the swarms, though polymorphic enough, are quite local. The hybrids themselves greatly resemble those of the *Solandri* × *truncata* group.

143. *N. fusca* × *truncata*.

144. *N. Solandri* × *truncata*.

*N. apiculata* (Col.) Ckn. is a name given to a very small portion of this group. The specimens in Colenso's herbarium seem to have been collected from one tree. Both the species have a wide range in the lowland belt and hybrid swarms are common, the individuals of which much resemble those of *cliffortioides* × *fusca*. Forms with closely-toothed leaves much smaller than those of either parent are a highly interesting feature.

#### MORACEAE.

145. *Paratrophis microphylla* × *opaca*.

Both parents are small bushy trees, but *P. microphylla* remains for many years a divaricating shrub. The hybrids show several intermediate life forms. *P. opaca* is a purely coastal tree, so that hybrid swarms appear only near the coast. In the absence of the other, each shows no sign of 'variability'. Allan (2) illustrates two forms.

#### SANTALACEAE.

146. *Mida myrtifolia* × *salicifolia*.

*M. eucalyptoides* A. Cunn. belongs to this group.

## LORANTHACEAE.

- 147.
- Elytranthe flavida*
- ×
- Colensoi*
- . D.

*E. flavida* has orange-yellow flowers, while those of *E. Colensoi* are scarlet. Forms of intermediate flower-colour (and leaf form) have been observed in *Nothofagus* forest in the north of South Island.

- 148.
- E. flavida*
- ×
- tetrapetala*
- .

This hybrid group is far from common, but the individual hybrids are well marked.

## POLYGONACEAE.

- 149.
- Muehlenbeckia australis*
- ×
- complexa*
- .

The group has been briefly discussed by Allan (5). In field studies the epharmony of both species must be taken into account.

- 150.
- M. axillaris*
- ×
- complexa*
- .

*M. axillaris* is a mat-shrub. *M. complexa* epharmonically either a liane or a bolster-like shrub with densely inter-tangled branches. The hybrids, so far as we have observed, have the bolster form, but show distinctly a blending of other characters.

- 151.
- M. axillaris*
- ×
- ephedroides*
- .

*M. muriculata* Col. belongs to this group. *M. ephedroides* has the leaves reduced to scales in the adult, and the hybrids might easily be mistaken for permanent juvenile forms.

- 152.
- M. complexa*
- ×
- ephedroides*
- .

So far only one or two hybrids of this group have been noted. Usually the two species get little chance of crossing.

## AIZOACEAE.

- 153.
- Mesembryanthemum aequilaterale*
- ×
- australe*
- . D.

*M. aequilaterale* is naturalized at Castle Point on the east coast of the North Island. It grows in company with the indigenous *M. australe*, and there also occur a number of intermediate forms, not seen where *M. australe*, grows alone.

## RANUNCULACEAE.

*Clematis*.

The New Zealand species of this genus have been extensively cultivated by Mr. S. Page, of Christchurch, who obtained plants from many parts of the region, and made numerous crossings. The hundreds of plants in his garden presented a striking polymorphy. His work showed how readily the species cross, but as the New Zealand species are dioecious no really decisive evidence as to parentage can be secured without special precautions.

154. *Clematis Colensoi* × *hexasepala*. D.

Usually the two species do not grow side by side, but the occurrence of intermediates when this happens is strongly suggestive of hybridism.

155. *C. foetida* × *hexasepala*.

But few examples have been observed, but these show clearly the result of the combination of the small yellow flowers of *foetida* with the larger white ones of *hexasepala*.

156. *C. hexasepala* × *indivisa*.

The two species do not usually grow together, so the occurrence of hybrids is infrequent.

*Ranunculus*.

A number of well-marked hybrid groups have already been distinguished in this genus, which should provide excellent material for detailed studies. Wall (61) has described a single remarkable plant to which he gives the name *R. huttensis*. He ascribes to it the origin *R. chordorhizos* × *Monroi* var. *dentatus*. There is little doubt that this view is correct.

157. *Ranunculus Buchanani* × *Lyallii*.

For a description of the group see Allan (5).

*R. Matthewsii* Cheesem. was described from a few specimens belonging to this group. This hybrid group affords a beautiful example of extensive hybridism over a limited area when a species of wide range meets one of much more restricted distribution. It was only on the discovery of such swarms that there became reason to dispute the status of *R. Matthewsii*.

158. *R. Buchanani* × *Scott-Thomsonii*.

See Allan (5). *R. Scott-Thomsonii* is a rare species confined to 'rock-slides', while the wider-ranging *R. Buchanani* is a plant of rock-cliffs. The hybrids mainly occur on the more stable debris between the two habitats.

159. *R. Buchanani* × *Simpsonii*.

Cockayne had one or two of these hybrids in cultivation more than forty years ago. Cheeseman, who saw dried specimens only, considered them (the colour of the flowers being lost) glabrous forms of *R. sericophyllus*, a plant which does not occur in the mountains whence Cockayne's specimens were secured.

160. *R. depressus* × *rivularis*.*R. Enysii*.

*R. Enysii* Kirk was described from specimens collected in the Castle Hill Basin (Eastern South Island). What there occurs is a series of unresolved jordanons and hybrids. The complexity is added to by the presence of forms of *R. Monroi* and *R. geraniifolius*, or a related species.

Hence *R. Enysii*, as seen in herbaria, includes diverse forms of uncertain status.

161. *R. foliosus* × *lappaceus* sens. lat.

162. *R. geraniifolius* × *Monroi*.

The two parents differ greatly, and the hybrids are readily recognizable. So far they have been collected only by Allan on Mount Trovatore, North-west Nelson, South Island.

163. *R. geraniifolius* × *nivicola*. D.

The two occasionally meet, but owing to the polymorphy of *R. geraniifolius* this hybrid group is suspected rather than known.

164. *R. geraniifolius* × *verticillatus*.

It was the recognition of the hybrids as such that lead to the conclusion, contrary to Cheeseman's opinion, that *R. verticillatus* is a valid species.

165. *R. gracilipes* × *Sinclairii*.

The two are rather jordanons of a compound species than true simple species.

166. *R. lappaceus* sens. lat. × *multiscapus*.

We include in this group a polymorphic series of forms that links up the two parents, the first-named of which is itself a compound species.

167. *R. macropus* × *rivularis*. D.

Both are aquatic or semi-aquatic species, and both epharmone greatly. Without experiment hybridism can only be suspected.

168. *R. sericophyllus* × *Simpsonii*.

*R. Simpsonii* has hitherto been merged with *R. sericophyllus*, since the crossing of the two produces a whole series of forms ranging from the villous *sericophyllus* to the glabrous *Simpsonii*. *R. Baughani* Petrie possibly belongs to the hybrid group. We base the above on observations made by Messrs. Simpson and Scott-Thomson. We know *R. sericophyllus* as a well-defined jordanon growing on consolidated stony debris in the central and northern parts of the Southern Alps, where *Simpsonii* does not occur.

#### WINTERACEAE.

169. *Wintera axillaris* × *colorata*.

It is possible that *W. colorata* consists of a northern and a southern (south of lat. 40°) jordanon. If so, it is the northern jordanon only that is concerned in this common cross.

## CRUCIFERAE.

170. *Cardamine heterophylla* × *uniflora*.

These hybrids seem to occur chiefly on the southern coast of South Island, where *C. uniflora* is a common species.

171. *Lepidium matau* × *karwarau*. D.

We have seen these species growing together in one locality, and intermediates were present. Further study is required to substantiate the group.

172. *Pachycladon glabrum* × *novae-zelandiae*.

The two jordanons are so close that they should be treated as forming a compound species. We list the group to emphasize the fact that the one 'passes insensibly' (25, p. 469) into the other owing to hybridism and not to epharmony.

## DROSERACEAE.

173. *Drosera Arcturi* × *stenopetala*. D.

This and the following are based on observations by Allan on the Ruahine Mountains, North Island. Further study is desirable.

174. *D. spathulata* × *stenopetala*. D.

## ESCALLONIACEAE.

175. *Quintinia acutifolia* × *serrata*.

*Q. serrata* is found only in North Island. So far as we have observed the 'numerous intermediates' (25, p. 484) of *Q. acutifolia* are only known from the same island. In South Island, in the forests to the west of the Southern Alps, *Q. acutifolia* frequently forms a large part of the undergrowth and is apparently quite constant.

## PITTOSPORACEAE.

176. *Pittosporum Colensoi* × *tenuifolium*.

It will take many years of critical garden experiments before this group is well understood. Both *Colensoi* and *tenuifolium* are complicated linneons. A step in the right direction has been the bringing together of many distinct forms of *P. tenuifolium* into the Wellington Open Air Museum.

How great the polymorphy in *Pittosporum* can be is shown by a hedge in the Open Air Museum, hardly any two plants being alike. Mr. Beddie of Petone, near Wellington, sent to Cockayne dozens of different-looking plants, collected in an indigenous-induced community, composed largely of the so-called species *tenuifolium*. The various forms fall into different categories, with the rough general distinction of a flat-leaved and a waved-leaved group. Some of these would have been referred in a herbarium to that most problematic of species *P. Buchanani*.

177. *P. crassifolium* × *tenuifolium*. D.

This cross is quite common in cultivation, but we lack definite field information as to its occurrence in nature.

178. *P. divaricatum* × *rigidum*. D.

We suggest this on the basis of herbarium material from the north-eastern part of South Island, but in the absence of field observations there remains some doubt.

179. *P. ellipticum* × *tenuifolium*. D.

*P. intermedium* Kirk, known only from one plant, may be of this origin.

180. *P. eugenioides* × *tenuifolium*. D.

Mrs. Martin considers that undoubtedly hybrid forms occur near Wellington, and that they are recognizable, *inter alia*, from carrying the characteristic scent of the leaves of *P. eugenioides* (popularly called Lemon-wood). It should be easy to establish the group, as the inflorescences and flower-colour are so different in the two species.

181. *P. fasciculatum* × *tenuifolium*.

The status of *P. fasciculatum*, however, is itself in doubt; it belongs to the *tenuifolium* linneon spoken of under No. 176.

182. *P. pimelcoides* × *reflexum*.

The parent species are confined to Kauri forest, and, when they occur together, as is frequently the case, typical polymorphic hybrid swarms are well represented.

#### CUNONIACEAE.

183. *Weinmannia racemosa* × *sylvicola*. D.

Observations are much required at the line where the southern *W. racemosa* meets the northern *W. sylvicola*. Our knowledge is insufficient to state definitely whether hybrids occur or not. In field studies the adults must be the special object of attention—the leaves of the adult plant are 3-foliolate in *sylvicola*—1-foliolate in *racemosa*.

#### ROSACEAE.

##### *Acaena*.

The first definite recognition of the occurrence of hybridism in the New Zealand Flora was the record of Buchanan (18) of the crossing of the Australian *A. ovina* with the indigenous *A. Sanguisorbæ*. Bitter (16, p. 297) described several hybrids between New Zealand species, but the material was collected in various European gardens. It is now known that hybridism is prevalent in wild communities, and our list gives a preliminary treatment merely. The sowing of seeds of suspected hybrids has always in our experience produced diverse offspring quite resembling forms occurring in nature.

184. *Acaena Buchanani* × *microphylla*.

This is one of the many cases where a species of limited distribution (*A. Buchanani*) is overlapped by one of far wider range, and in the overlapped area occur large hybrid swarms.

185. *A. depressa* × *novae-zelandiae*.

Fine swarms occur on dunes bordering Foveaux Strait; whether the jordanon concerned is really *A. depressa* Kirk we are not sure.

186. *A. glabra* × *Sanguisorbae* var. *pilosa*.

*A. glabra* is almost confined to stony debris slopes ('shingle-slips') of high mountains, but occasionally gets a footing on more stable ground near the margins of the moving debris. Meeting there *A. Sanguisorbae* var. *pilosa* it hybridizes—the hybrids, easily recognizable by the fruiting heads, being no longer absolutely without hooked spines.

187. *A. inermis* × *microphylla*.

*A. microphylla* has hooked spines on the calyces, *A. inermis* is spineless; in the hybrids there is every transition from the spineless to the hooked spiny state. The swarms are widespread and of great diversity.

188. *A. inermis* × *Sanguisorbae*.

*A. inermis* crosses with several jordanons of *Sanguisorbae*, and swarms are widespread. Seeds sown from a hybrid with var. *pilosa* produced many forms similar to those found in nature.

189. *A. microphylla* × *Sanguisorbae*.

This is another widespread group.

190. *A. novae-zelandiae* × *ovina*.

Here the cross is between an indigenous and an exotic species that is common in many parts of the region.

191. *A. novae-zelandiae* × *Sanguisorbae*.

The red colour of the spines of *A. novae-zelandiae* is reduced in the hybrids by all stages up to its complete absence. Swarms with the var. *pusilla* of *Sanguisorbae* are especially common.

192. *A. ovina* × *microphylla*.

The species do not often meet, and hybrid swarms have only rarely been observed.

193. *A. ovina* × *Sanguisorbae*.

Hybrid groups are known between *A. ovina* and the vars. *pusilla* and *viridior* of *Sanguisorbae*. As for var. *viridior*, a very distinct jordanon, we have never seen, curiously enough, hybrids between it and var. *pusilla*, though both often grow in close proximity.

194. *A. Sanguisorbae* var. *pusilla* × var. *pilosa*.

So very distinct are the varieties in question, that the cross deserves mention. Many taxonomists would give them specific rank.

195. *A. Sanguisorbæ* var. *pilosa* × var. *sericei-nitens*.

The same remarks apply as in 194. The group has its headquarters in the southern part of South Island.

*Rubus*.

Examination of the types of the New Zealand species by Allan has revealed that for the most part the names have been incorrectly applied by local botanists, but here we retain the traditional usage as given by Cheeseman (25, p. 499). The species do not appear to hybridize very freely, and the hybrid forms so far observed are rare and local. They appear to be vegetatively vigorous, but highly sterile. All the species are dioecious.

196. *Rubus australis* × *parvus*.

*R. australis*, in one or other of its jordanons, ranges throughout the main islands, but *R. parvus* is confined to the western side of South Island. The two are extremely distinct in life form. *R. parvus* is a creeping and rooting mat-plant with 1-foliolate leaves and solitary flowers; *R. australis* is a large, high-climbing liane, with 3-5-foliolate leaves and large panicles. In 1898 a single hybrid plant was discovered by the late Mr. S. D. Barker. This exceedingly handsome hybrid is now cultivated in many gardens, all the plants having descended by vegetative propagation from the original wild plant. Only twice since then have plants been observed to flower. For further discussion of × *R. Barkeri* see Cockayne (26), and Allan (7). More recently another form was discovered by Dr. W. Mackay of Greymouth. This has a considerable resemblance to *R. Barkeri*, but flowers and fruits regularly.

197. *R. cissooides* × *schmidelioides*. D.

The late Mr. Carse collected specimens that he doubtfully attributed to this origin.

198. *R. parvus* × *schmidelioides* var. *coloratus*.

Allan (7, 10), has raised an artificial cross of this parentage. The plants are almost exactly identical with ones that we believe to have been found wild, and for which prior to the cross we had assigned the origin here stated.

199. *R. schmidelioides* var. *coloratus* × *subpauperatus*.

We here apply the name *coloratus*, perhaps incorrectly, to a jordanon common in the montane belt of South Island closely related to *R. subpauperatus*; indeed, it is best to consider it a broad-leaved variety of that well-marked species.

LEGUMINOSAE.

200. *Carmichaelia Monroii* × *subulata*. D.

We place here certain plants that bear a close resemblance to the



rigid-stemmed, dwarf *C. Monroii*, but are of considerably greater stature. One of these was labelled *C. humilis* in a collection of dried specimens given by Petrie to Cockayne.

201. *C. robusta* × *subulata*. D.

There appear to be many forms which come more or less midway between *C. subulata* and a form of *C. robusta* common in certain parts of the central montane belt of the eastern ranges of the Southern Alps.

202. *Edwardsia chathamica* × *microphylla*. D.

*E. chathamica*, or a very closely related jordanon, occurs on the mainland as well as on Chatham Island. It differs from *E. microphylla* in the much less divaricating juvenile form, and in other characters, but appears to hybridize with it.

203. *E. chathamica* × *tetraptera*. D.

*E. tetraptera* is confined to the East Cape botanical district of North Island, but on its margins meets the mainland form of *E. chathamica* and appears to hybridize with it. Whether this be so or not, it is certain that there are many distinct forms of *Edwardsia* that cannot be referred to any of the described species. Some of these, doubtless, are jordanons, but it is likely that there are also several hybrid groups.

204. *E. microphylla* × *prostrata*.

A considerable range of forms is produced by this cross, some of which have been brought into cultivation. Although the usual habitat of *E. prostrata*, dry stony or rocky ground, exposed to much sun and wind, certainly has some influence on the stature of individuals, Cockayne found that the species comes true from seed.

#### GERANIACEAE.

205. *Geranium dissectum* var. *glabratum* × *pilosum*.

This is given on the authority of Carse, who paid much attention to the group, and is supported by the series of specimens collected by him.

#### LINACEAE.

206. *Linum marginale* × *monogynum*. D.

*L. marginale* is a blue-flowered species introduced from Australia. It sometimes is found in association with the white-flowered indigenous *monogynum*. In such a community Allan has observed individuals with variously flecked flowers. But there is a jordanon of *L. monogynum* with blue-striped flowers, occurring on Chatham Island and to a limited extent near Cook Strait. Until progeny have been secured no certainty can be arrived at.

## RUTACEAE.

207. *Melicope simplex* × *ternata*.

*M. Mantellii* Buch. forms part of this group, for an account of which see Allan (2). The hybrids show a complete range from the bushy tree form of *M. ternata* to the divaricating shrub form of *M. simplex*. Since *M. Mantellii* was applied to a few forms quite midway between the parents, it seems to us wrong to extend that name to cover the whole group.

## CORIARIACEAE.

The genus *Coriaria* was early famous in New Zealand botany for the multiplicity of forms existing. A good deal of work has been accomplished in analyzing the group in the light of hybridism, but much field and cultural work yet remains before a thorough understanding of the jordanons involved can be reached. To take one example—no species in the flora seems better marked than the tall woody *C. arborea*, but it is quite possible that the truly herbaceous *C. sarmentosa*, the above-ground parts of which die to the ground yearly, may be merely a plant that will not tolerate much frost, and that were such absent it would develop a permanent erect main stem. Until experiments have been made no certain conclusions can be drawn.

208. *Coriaria angustissima* × *lurida*.

*C. lurida* as used by us refers to a compound species containing several distinct jordanons. Whether *C. lurida* Kirk refers to a jordanon or a hybrid cannot be determined from the material available—a dried specimen in Kirk's herbarium. The specimen is certainly very close in appearance to what we consider from field evidence to be a jordanon.

209. *C. angustissima* × *sarmentosa*.

The swarms are large and the polymorphy very great. See Allan (8).

210. *C. arborea* × *lurida*.

The Mount Egmont jordanon referred to by Allan (8) to *C. sarmentosa* should rather be placed under *C. arborea*, treating that as a compound species. Some of the hybrids between *C. arborea* and *C. lurida* var. *undulata* are illustrated by Allan (13).

211. *C. arborea* × *sarmentosa*. D.

As shown above, cultural experiments are required in order to define the range of epharmonic plasticity of these species.

212. *C. lurida* × *sarmentosa*.

= × *C. sarlurida* Ckn. et Allan.

## ELAEOCARPACEAE.

213. *Aristotelia fruticosa* × *serrata*.

The group is discussed by Allan (8). *A. Colensoi* Hook. f., as the type material shows, was based on specimens without flowers approaching rather closely the *serrata* parent. The group is very large and highly polymorphic. The only confusion that can arise is owing to the great resemblance of some forms of juvenile *A. fruticosa* to some of the hybrid forms. It is interesting to note that at one point within the range of these two species a river valley rather more than a mile wide, at the junction of a very wet and a moderately dry climate, separates an area, where *A. fruticosa* alone grows, from a forest that contains both species together with dozens of different hybrids.

214. *Elaeocarpus dentatus* × *Hookerianus*.

Our knowledge of this group is due to the painstaking work of Mr. A. D. Beddie, who has collected a number of interesting forms from swarms in the southern part of North Island.

Mr. Beddie points out that Plate No. 12 in Kirk's *Forest Flora*, which is labelled '*Elaeocarpus Hookerianus*, young state', is not at all like the juvenile of that species, but matches closely one of the wild hybrids.

## MALVACEAE.

215. *Hoheria angustifolia* × *Lyallii*.

The first specimens collected were described as *Gaya Allanii* by Cockayne (36), only a few examples being discovered. Later a swarm was discovered by Allan, revealing that *G. Allanii* was only one member of a polymorphic group. The two species belong to different sections of the genus, one of which has been referred to no less than three different genera. Thus the cross is almost intergeneric.

216. *H. angustifolia* × *sexstylosa*.

The two species have similar habitat requirements, frequently meet, and produce very polymorphic swarms. See Allan (4).

217. *Plagianthus betulinus* × *divaricatus*.

*P. cymosus* Kirk was based (54, p. 71) on 'very limited' material taken from a cultivated plant, and in the absence of 'intermediates' hybridism was not suspected. Subsequent examination showed that where the species meet great swarms occur—see Cockayne and Allan (36). The group is especially interesting owing to the extreme diversity of the parents in life form and habitat requirements. *P. betulinus* is a tall, deciduous canopy tree of lowland forest, *P. divaricatus* a divaricating twiggy shrub of salt swamp. Only where a tidal river invades lowland forest can the two species meet.

218. *P. chathamica* × *divaricata*.

Specimens of this cross collected by Mr. W. Martin, and others by Mr. K. W. Dalrymple, in Chatham Island, would by a systematist not recognizing wild hybridism be placed under *P. cymosus* Kirk.

VIOLACEAE.

219. *Hymenanthera crassifolia* × *obovata*.

For a discussion see Allan (8).

220. *Melicytus lanceolatus* × *ramiflorus*.

See Allan (9).

221. *M. micranthus* var. *longiusculus* × var. *microphyllus*.

The var. *microphyllus* is a very distinct jordanon; the limits of *longiusculus* are vague, and certain forms placed under that name may really be *microphyllus* × *ramiflorus*.

THYMELAEACEAE.

222. *Drapetes Dieffenbachii* × *villosa*.

The polymorphy of the two species is badly known, hence the status of many forms found in herbaria is not clear.

223. *Pimelea arenaria* × *prostrata*. D.

*P. arenaria* is a sand-dune species, and sometimes meets a sand-hollow jordanon of the polymorphic *P. prostrata*. Forms suggestive of hybridism have been observed, but the two species do not appear to cross readily, so that breeding tests of the supposed hybrids are needed.

224. *P. aridula* × *prostrata*. D.

There appear to be in various localities a good many intermediate forms requiring investigation.

225. *P. buxifolia* × *prostrata*. D.

226. *P. Gnidia* × *longifolia*.

Cheeseman's *P. Gnidia* var. *pulchella* is almost certainly a form of this group. In one locality in the north-west of South Island where forest has been burned a huge swarm of these hybrids, together with the parents and a great number of *Hebe* hybrids, are now in evidence.

227. *P. Gnidia* × *prostrata*. D.

Carse collected specimens that possibly come here.

228. *P. prostrata* × *Lyallii*.

The species grow together on the shores of Foveaux Strait, and these hybrid forms are common, showing considerable diversity among themselves. We limit *P. Lyallii* to one or more coastal jordanons.

229. *P. prostrata* × *pseudo-Lyallii*.

Under the name *pseudo-Lyallii* we place that mixture of alpine and subalpine jordanons included by Cheeseman with the coastal *P. Lyallii* Hook. f. The group is thus not yet well defined.

230. *P. prostrata* × *sericeo-villosa*.

Hybrids between these extremely distinct species, a mat-plant (*prostrata*) and a small hairy cushion (*sericeo-villosa*), are readily recognizable in the field.

231. *P. prostrata* × *Traversii*. D.

## MYRTACEAE.

*Leptospermum*.

The polymorphy in *Leptospermum* is extreme and renders detailed taxonomic study of the genus very difficult, although the two compound species *L. ericoides* and *L. scoparium* are quite distinct from each other. In many parts of the region communities of *L. scoparium* possibly contain nothing but hybrids, the jordanons being swamped out.

232. *L. ericoides* × *lineatum*.

We have noted these hybrids on sand dunes in the far north of North Island, where *L. lineatum* is a common and well-marked species.

233. *L. ericoides* × *scoparium*. D.

The two species are so ultra-polymorphic that it is impossible certainly to ascribe hybridism to the intermediate forms seen without crossing experiments.

234. *Metrosideros Colensoi* × *hypericifolia*.

*M. Colensoi*, though of fairly wide range, is extremely local, so that hybrids are not common.

235. *M. robusta* × *tomentosa*.

Carse (21) published × *M. subtomentosa* with a composite description based on three individuals differing in certain details. Oliver (57) points out that Kirk's var. *intermedia* of *robusta* was also based on a hybrid specimen.

236. *Myrtus bullata* × *obcordata*.

For details of the polymorphy of this group, which includes *M. Ralphii* Hook. f., see Cockayne (29) and Allan (11). In the latter paper it was shown that seed from a single hybrid produced forms ranging all the way from forms indistinguishable from *M. obcordata* to some very close indeed to *M. bullata*. In some places large hybrid swarms have come into being after the destruction of forest.

## ONAGRACEAE.

Focke (46, p. 162) refers to *Epilobium* hybrids recognized by Haussknecht in New Zealand material, that were dealt with in the latter's then unpublished monograph. Focke (46, p. 165) also refers to polymorphy in the New Zealand *Fuchsiae*. We are still far from an adequate knowledge of the extent of hybridism in *Epilobium*, and the present list will certainly be amplified when detailed studies are made.

237. *Epilobium Billardierianum* × *erectum*.

238. *E. Billardierianum* × *junceum*.

239. *E. Billardierianum* × *pubens*.

240. *E. chionanthum* × *erectum*.

241. *E. erectum* × *hirtigerum*.

242. *E. erectum* × *junceum*.

243. *E. erectum* × *pallidiflorum*.

244. *E. glabellum* × *junceum*.

245. *E. glabellum* × *novae-zelandiae*.

246. *E. glabellum* × *pubens*.

Kirk's *E. perplexum* (54, p. 170) is probably in part based on this cross, but Cheeseman (25, p. 607) places it under the very distinct *E. chloraeifolium*.

247. *E. hirtigerum* × *junceum*.

248. *E. junceum* × *pallidiflorum*.

249. *E. linnaeoides* × *rotundifolium*.

The two species very closely resemble each other, but occasion no difficulty in identification when growing apart.

*E. tasmanicum*.

This, so far as New Zealand material is concerned, is a name for a group of forms that have never been closely studied. There are probably several jordanons, some of which may be worthy of specific rank.

250. *Fuchsia excorticata* × *perscandens*.

See Allan (9) for a discussion of the group. An examination of the type specimens of *F. Colensoi* Hook. f. shows that it was based on hybrid material approaching the *perscandens* parent. In the *F. procumbens* covers at Kew are some flowerless pieces of the true *F. perscandens*, which is also represented in a sheet collected by A. Cunningham. The group is noteworthy since the cross is between a massive tree and a slender scrambling liane. In certain forests where the undergrowth has been destroyed by cattle are now large swarms of this group.

## ARALIACEAE.

251. *Nothopanax anomalum* × *simplex*.

This group, and the status of *N. parvum* Kirk, are discussed by Cockayne and Allan (36), and Allan (4). This supplies another example of hybrid swarms being increased through the destruction of forest, and more light being available.

252. *N. arboreum* × *Colensoi*.

An important feature of this hybrid group is the transition in length of petiolules from the sessile (*Colensoi*) to the well-stalked (*arboreum*) condition.

253. *N. arboreum* × *laetum*.

*N. laetum* has very large leaves; the hybrids show all sizes between the parents.

254. *N. arboreum* × *simplex*.

This has been observed in north-western South Island. Probably *N. Macintyreii* Cheesem. belongs to this group.

255. *N. Colensoi* × *simplex*.

The known occurrences of this are few, in the north of South Island.

256. *N. Edgerleyi* × *Sinclairii*. D.

Allan has noted certain intermediate forms on Mount Egmont, North Island.

257. *N. arboreum* × *Pseudopanax crassifolium* var. *unifoliolatum*. D.

On Kapiti Island, off the west coast of Wellington, occurs a polymorphic series of seedlings in many places in damaged forest where the species grow together (36). Mr. Beddie has found many forms near Wellington corresponding to those of Kapiti Island.

258. *Pseudopanax crassifolium* × *ferox*. D.

Mr. C. M. Smith collected a remarkable juvenile form on the track to Preservation Inlet, west coast of South Island, which may belong to this cross.

259. *P. crassifolium* × *Lessonii*.

This is a group of considerable diversity. It may be that *P. crassifolium* var. *trifoliolatum* is one of the hybrids. Cockayne is greatly indebted to His Lordship Bishop Williams for studying the matter in the eastern part of North Island, and sending him many beautiful seedlings for cultivation.

## UMBELLIFERAE.

260. *Aciphylla Colensoi* × *Scott-Thomsonii*.

According to Messrs. Simpson and Thomson hybrids between these specimens are not uncommon.

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261. *A. Colensoi* × *Simpsonii*.

We admit this group on the authority of Messrs. Simpson and Thomson, who have devoted close study to wild hybrids, and have cultivated many in their gardens.

262. *A. congesta* × *Cuthbertiana*. D.

263. *A. conspicua* × *intermedia*.

Recent collections on the Tararua Mountains by Mr. N. Elder appear to put this group beyond doubt.

264. *A. crenulata* × ? *conspicua*.

From Mr. R. M. Laing we have received a number of *Aciphyllae* that match no known species but come from the neighbourhood of Arthur's Pass, South Island, where the *Aciphylla* jordanons are fairly well known. Cockayne also collected there certain anomalous forms. That hybridism is more or less common in that locality seems assured, but whether we are here adopting the correct parentage remains to be proved.

265. *A. crenulata* × *similis*.

This is one of the groups referred to under No. 264.

266. *Angelica decipiens* × *montana*.

Where these two species come together—a by no means common occurrence—polymorphic individuals occur, which are absent elsewhere. It may be that *A. peiraea* also crosses with *A. montana*, as the resulting forms would be closely similar to those mentioned.

267. *Angelica montana* × *trifoliolata*. D.

Specimens collected by Miss L. M. Cranwell in the type locality of *A. trifoliolata* suggest hybridism strongly, but, as epharmony may be a partial explanation of the diversity shown, further examination is desirable.

268. *Anisotome antipoda* × *latifolia*.

*A. latifolia* var. *angustata* Kirk comes into this group, which was studied in the field by Cockayne on the Lord Auckland Islands.

269. *A. aromatica* × *Haastii*. D.

On Arthur's Pass there is a distinct form of *Anisotome* not so far seen elsewhere, which may be either an undescribed jordanon, or a hybrid (presumably  $F_1$ ) as suggested above. An objection to the hybridization hypothesis is that similar forms have not been observed elsewhere, though the two species frequently meet.

270. *A. capillifolia* × *Haastii*.

This is another example of a species of wide distribution (*Haastii*) meeting one of much more restricted limits.



271. *A. Haastii* × *pilifera*.

Included in this group is *A. pilifera* var. *pinnatifida* Kirk. The cross is not common, nor is great diversity shown in the hybrids. The fertility of *Anisotome* crosses has not been tested.

272. *Apium filiforme* × *prostratum*.

*A. filiforme* at times occurs inland far from the sea, and in such cases hybrid forms are absent. Where the two meet on the coast hybrids are common.

273. *Hydrocotyle dissecta* × *elongata*.

This cross has so far been detected only in one locality in northern South Island, but the contrasting characters of the parents make the recognition of the hybrid forms easy.

274. *Oreomyrrhis Colensoi* × *ramosa*.

The two species are well marked and hybrids are not infrequent. There are probably other *Oreomyrrhis* hybrids, but the limits of some of the jordanons have not been clearly worked out.

## CORNCEAE.

275. *Corokia buddleoides* × *Cotoneaster*.

The group is briefly discussed by Allan (4). *C. Cheesemanii* Carse was described from a portion of a polymorphic series that Carse later recognized as a hybrid swarm. The two species only come together in a few localities and there alone the hybrids are found.

## ERICACEAE.

*Gaultheria*.

Kirk in 1873 (52) suggested that *G. fagifolia* Hook. f. might be a hybrid, but even as late as 1925 Cheeseman (25, p. 591) merely remarks 'This appears to me a mere form of *G. rupestris*, verging towards *G. oppositifolia*, or possibly a hybrid between the two plants'. Since then it has been shown conclusively that hybridism is extremely common in the genus, and all the species are concerned. Sir Arthur Hill commenced a detailed study of the whole question while in New Zealand, and has since been supplied with a wealth of material from many localities.

276. *Gaultheria antipoda* × *oppositifolia*.

One jordanon of *G. antipoda* extends through the main islands. In the centre of North Island, where the surface soil is mainly pumice, it meets the beautiful *G. oppositifolia*, and a wealth of polymorphic hybrids appears. Some examples bear on the same plant both the succulent calyces of *antipoda* and the dry ones of *oppositifolia*.

277. *G. antipoda* × *perplexa*.

*G. antipoda* is an erect, bushy, small shrub, with a capsular fruit;

*G. perplexa* is a spreading, prostrate shrub, with a berry-like fruit. In the hybrids all sorts of combinations of these characters are evident.

278. *G. antipoda* × *rupestris*.

*G. rupestris* occurs in several well-marked geographical jordanons, so that more than one hybrid group is represented by the formulae in which *rupestris* is included.

279. *G. depressa* × *perplexa*.

280. *G. depressa* × *rupestris*.

The influence of the strongly swollen calyx of *G. depressa* is seen in many of the hybrids.

281. *G. oppositifolia* × *rupestris*.

The two do not often meet, but hybridize freely when they do.

#### EPACRIDACEAE.

282. *Dracophyllum filifolium* × *recurvum*.

= *D. arcuatum* W. R. B. Oliv.

The short recurved leaves of *D. recurvum* are very distinct from the long needle-like leaves of *D. filifolium*, while the life form of each is also very different, so that the hybrids are for the most part easily recognizable. Oliver (58) gives very short diagnoses for the hybrid groups named by him, but remarks 'it must be remembered that a series of hybrids between any two species may include forms grading into both parents'.

283. *D. filifolium* × *subulatum*.

× *D. vulcanicum* W. R. B. Oliv.

284. *D. Kirkii* × *prorum*.

= × *D. saxicolum* W. R. B. Oliv.

285. *D. Kirkii* × *uniflorum*.

We have seen a fair number of these hybrids in the Arthur's Pass area, the contrasting characters of the two parents make the hybrids readily recognizable.

286. *D. Lessonianum* × *squarrosum*.

= × *D. densiflorum* W. R. B. Oliv.

287. *D. Lessonianum* × *subulatum*.

= × *D. marginatum* W. R. B. Oliv.

288. *D. longifolium* × *politum*.

We think that into this group comes *D. Pearsoni* Kirk, and that this may perhaps represent an F<sub>1</sub> hybrid.

289. *D. longifolium* × *prostratum*.

On the mountain bogs of the south of South Island several species of *Dracophyllum* often occur together, so that a confusing array of forms may be collected not at all easy to analyse.

290. *D. longifolium* × *recurvum*.  
= × *D. varium* W. R. B. Oliv.

291. *D. longifolium* × *scoparium*.  
= × *D. insulare* W. R. B. Oliv.

292. *D. longifolium* × *Traversii*.

Hybrids of this origin have been observed by Allan on Arthur's Pass, and on the mountains near the Franz Josef and the Fox Glaciers. The hybrids were not plentiful, and had much the habit of *Traversii* with foliage much nearer to that of *longifolium*.

293. *D. longifolium* × *uniflorum*.

Oliver (58), on the authority of Wall, who had studied the type specimen of *Epacris rosmarinifolia* Forst., considers *D. uniflorum* Hook. f. an absolute synonym. Allan has also seen Forster's type and agrees that it probably (it is a mere fragment) belongs to the same compound species as *D. uniflorum* Hook. f. But since Hooker's type presents certain differences in detail, and is the form we know as crossing with *D. longifolium* we retain Hooker's name in the meantime. Oliver (58) considers *D. acicularifolium* Ckn. to belong to the hybrid series and adopts this name for the group. No doubt some of the hybrids closely mimic *D. acicularifolium* and are included under that name in herbaria, but there is very strong field evidence that there is a jordanon corresponding to Cockayne's conception of *D. acicularifolium*, a view recently confirmed by Messrs. Simpson and Thomson.

294. *D. prostratum* × *uniflorum*.

This is one of the groups found growing on the mountain bogs of the south of South Island already referred to.

#### MYRSINACEAE.

295. *Suttonia australis* × *divaricata*.

A single example of this group was recently discovered by Miss M. Finlayson at Puerua in South Otago. This at once raised the question of the status of *S. montana* Hook. f. Miss L. M. Cranwell, Botanist at the Auckland Museum, on examining the material in Cheeseman's and in Petrie's herbaria, found that there was no constancy in the forms placed under *S. montana*. The whole series is strongly suggestive of the above hybrid group, and *Myrsine neo-zealandensis* appears to be one striking form of it. While hybridism cannot be certainly established from herbarium material that lacks adequate field notes, the fact that no one specimen matches another leaves but little doubt on the question.

## OLEACEAE.

296. *Olea Cunninghamii* × *lanceolata*.

The species are common in the south of North Island, and hybrids appear to be not uncommon in damaged forest.

## GENTIANACEAE.

*Gentiana*.

Cheeseman (25, p. 724) has well said of the genus in New Zealand, 'a bewildering multitude of closely allied forms, to arrange which systematically is a most perplexing task.' While the clue of hybridism will greatly help to solve the puzzles, it is also clear that epharmony plays a great part. The difficulty of coming to clear-cut decisions is greatly increased by the refusal of the species in general to be cultivated. To succeed with this two methods are available: (1) take up a small plant together with a large amount of soil and place this in a suitable spot in a well-constructed alpine garden; (2) sow seed in situ in such a garden.

297. *Gentiana bellidifolia* × *corymbifera*.298. *G. bellidifolia* × *divisa*.299. *G. bellidifolia* × *patula*. D.

It may be that we have here a series of epharmones and not a hybrid group. We do not think anybody in a majority of cases can distinguish the one so-called species from the other. It may be done in herbaria, but we doubt if it can be done in the field.

300. *G. cerina* × *concinna*.

Any one who has visited the Lord Auckland Islands in summer should have little doubt as to the occurrence of this hybrid group, so varied in the colour of the flowers (crimson to almost white).

301. *G. Grisebachii* × *bellidifolia*. D.

The limits of *G. Grisebachii* are very vague. As the name is used by local botanists it certainly refers to a mixture of jordanons, epharmones, and hybrids.

## APOCYNACEAE.

302. *Parsonsia capsularis* × *heterophylla*.

See Allan (5). Both species are compound, the var. *rosea* of *capsularis* being especially well-marked, its hybrids showing varied colour forms. The status of Carse's *parvifolia* and *grandiflora* of *P. capsularis* is uncertain.

## CONVOLVULACEAE.

303. *Calystegia Soldanella* × *tuguriorum*. D.

Intermediates between the prostrate *C. Soldanella* and the climbing *tuguriorum* are sometimes met with, but the case needs further study.

## BORAGINACEAE.

*Myosotis australis*.

The name is applied to an un-analysed linneon containing white-flowered and yellow-flowered forms, none of which appear to be conspecific with the Tasmanian plant to which the name *australis* was first given.

304. *M. macrantha* × *Traversii*.

Particularly fine swarms of this group occur on abandoned stream-beds at the foot of Mount Sefton in the Mount Cook area. *M. Traversii* has lemon-yellow flowers, *M. macrantha* brownish-orange. The hybrids show a great range of colour, including deep bronze and white.

*M. pygmaea*.

*M. pygmaea* is a polymorphic linneon, and until it is properly analysed the systematic status of its members cannot be estimated.

## SCROPHULARIACEAE.

305. *Euphrasia Cockayneana* × *revoluta*.306. *E. Cockayneana* × *zelandica*.

The two species are closely allied, but *E. Cockayneana* has bright yellow flowers, *E. zelandica* white. The hybrids show a range of colour-forms.

307. *E. cuneata* × *tricolor*. D.

The extent to which the intermediate forms are due to hybridism or to epharmony has not yet been worked out in the field.

*Hebe*.

Hooker (50, p. 190) stated 'the species hybridize with great facility', but he referred rather to garden than to wild hybrids. Cheeseman (25, p. 777) says 'several species hybridize so readily in cultivation that the supposition at once arises that natural hybrids may occur'. He diagnoses eighty-six species. In our revisionary paper (38) we showed that at least twenty of these are hybrid groups or unresolved linneons. Here we list forty-three wild hybrid groups, and if we err, we feel that it is on the side of caution.

308. *Hebe Allanii* × *amplexicaulis*.309. *H. angustifolia* × *leiophylla*.

310. *H. angustifolia* × *salicifolia*.

= × *H. angustisala* Ckn. et Allan.

Both parents are compound, *H. salicifolia* markedly so; therefore several hybrid groups are included. × *H. Simmondsii* Ckn. is one form of the group *H. angustifolia* × *salicifolia* var. *Atkinsonii*.

*H. gracillima* (Cheesem.) Ckn. derives from *H. angustifolia* × *salicifolia* var. *communis*.

311. *H. Astoni* × *buxifolia*.

This group, characteristic of the forms produced when a 'whipcord' *Hebe* crosses with one of the small-leaved species, is illustrated by Allan (9). The hybrids mimic rather closely the juvenile stage of *H. Astoni*, and at one time were considered as persistent juveniles by Cockayne.

312. *H. Astoni* × *laevis*.

= × *H. laevastoni* Ckn. et Allan.

The hybrids are similar to those of No. 311, and could hardly be distinguished from such by means of herbarium specimens only.

313. *H. Buchanani* × *buxifolia*.

314. *H. Buchanani* × *pinguifolia*.

Cheeseman's var. *major* of *H. Buchanani* belongs to this group.

315. *H. buxifolia* × *Hectori*.

316. *H. buxifolia* × *laevis*.

The species are closely allied, but the much denser and shorter inflorescences of the hybrids distinguish them from *laevis*.

317. *H. buxifolia* × *lycopodioides*.

*H. cassinioides* (Petrie) Ckn. is a form of this group. Petrie based his species on wild and cultivated plants, which probably belonged to different groups. Cockayne originally considered it a species with a persistent juvenile form. In New Zealand nurseries similar hybrids are being sold under the name *H. Laingii*—a distinct whipcord *Hebe* from Stewart Island not yet in cultivation.

318. *H. buxifolia* × *Menziesii*.

319. *H. buxifolia* × *Scott-Thomsonii* sp. nov. ined.

*H. Scott-Thomsonii* is found in the Otago Lake District, but as yet we have had no opportunity of describing it.

320. *H. buxifolia* × *tetragona*.

321. *H. buxifolia* × *vernica*.

322. *H. chathamica* × *Dieffenbachii*.

323. *H. Cookiana* × *salicifolia*.

324. *H. dasyphylla* × *uniflora*.

325. *H. Dieffenbachii* × *Dorrien-Smithii*.

Apparently all the species of Chatham Island except *H. gigantea* (a true tree) cross freely, and *Hebe* polymorphy is extreme. *H. Dorrien-Smithii*, as at present understood, is undoubtedly a linneon.

326. *H. elliptica* × *salicifolia*.

= × *H. ellipsala*. Ckn. et Allan.

A somewhat detailed study of a particular swarm belonging to this group has been given by Allan, Simpson, and Thomson (14). *H. amabilis* (Cheesem.) Ckn. and its var. *blanda* Cheesem. belong to a limited portion of the group.

327. *H. epacridea* × *Haastii*.328. *H. glaucophylla* × *leiophylla*.329. *H. glaucophylla* × *Traversii*.330. *H. Haastii* × *macrocalyx*. D.331. *H. laevis* × *salicifolia*.

= × *H. laevisala* Ckn. et Allan.

*H. Carsei* (Petrie) Ckn. is a mixture of forms belonging to this group.

332. *H. laevis* × *tetragona*.

Only a few forms of this group have been collected, but of their hybridity there can be no doubt. Some are in cultivation in the Wellington Open Air Museum.

333. *H. latisejala* × *macrocarpa*.

These show many flower-colours, from bright pink to white.

334. *H. leiophylla* × *salicifolia*.

= × *H. leiosala* Ckn. et Allan.

*H. Kirkii* (J. B. Armstrong) Ckn. is a form of this group with *H. salicifolia* var. *communis* as a parent.

335. *H. leiophylla* × *Traversii*.336. *H. ligustrifolia* × *salicifolia*.337. *H. macrocarpa* × *salicifolia*.

= × *H. macrosala* Ckn. et Allan.

Cheeseman's var. *affinis* of *H. macrocarpa* belongs here.

338. *H. macroura* × *salicifolia*.

*H. divergens* (Cheesem.) Ckn. is a form of the group.

339. *H. Menziesii* × *salicifolia*.340. *H. Menziesii* × *vernica*.341. *H. montana* × *pinguifolia*.

Where *H. Traversii*, *H. montana*, and *H. pinguifolia* grow in close proximity hybrid swarms of the utmost complexity are formed, the

relationships of which cannot be unravelled without genetic study. A good many forms are in cultivation in the Wellington Open Air Museum.

342. *H. montana* × *Traversii*.

*H. montana* is a name applied to several jordanons, so that the formula given refers to more than one hybrid group.

343. *H. obtusata* × *macrocarpa*.

344. *H. obtusata* × *salicifolia*.

× *Veronica Bishopiana* belongs to this group. See Allan (9).

345. *H. parviflora* × *salicifolia*.

This and other groups are now being studied cytologically by Dr. O. H. Frankel.

346. *H. pimeleoides* var. *rupestris* × *salicifolia*.

One of the forms has been named *H. Dartoni* (Petrie) Ckn.

347. *H. pubescens* × *salicifolia*.

A fine collection of this group was made by Mr. C. E. Christensen at Mercury Bay (east coast of North Island), proving definitely its hybrid origin.

348. *H. salicifolia* × *speciosa*.

The species seldom meet in nature, but hybrid forms have been observed by Allan in one locality on the west coast of North Island. A large series of garden hybrids has been raised by means of this cross.

349. *H. salicifolia* × *subalpina*.

350. *H. salicifolia* × *Traversii*.

351. *H. subalpina* × *Willcoxii*.

It is very probable that *H. Willcoxii* is a synonym of *H. Cockayniana*.

352. *Jovellana repens* × *Sinclairii*.

353. *Ourisia caespitosa* × *glandulosa*.

354. *O. caespitosa* × *prorepens*.

355. *O. Cockayniana* × *prorepens*.

356. *O. Colensoi* × *macrophylla*.

*O. Colensoi* is a small, pilose species of much more restricted distribution than the larger, more glabrous, *O. macrophylla*. In certain localities hybrids are common.

357. *Veronica diffusa* × *lanceolata*.

358. *V. diffusa* × *Lyallii*.

359. *V. Lyallii* × *Hebe*?

*V. loganioides* J. B. Armstr., collected by Armstrong in South Island, is a remarkable hybrid between a whipcord *Hebe* and *Veronica Lyallii*. Without field study it is impossible to say what actual species of *Hebe* is



concerned. It has the habit of a whipcord *Hebe* cross with the capsule and flower of a *Veronica*.

## PLANTAGINACEAE.

360. *Plantago Brownii* × *lanigera*.

361. *P. Brownii* × *spathulata*.

## RUBIACEAE.

*Coprosma*.

The flowering period is long drawn out in many species, and the flowering dates given in floras are approximate only, so that there is much more opportunity to cross than these indicate.

362. *Coprosma arborea* × *spathulata*.

363. *C. arcolata* × *rotundifolia*.

364. *C. Astoni* × *pseudo-Colensoi*.

365. *C. Astoni* × *foetidissima*.

366. *C. Colensoi* × *foetidissima*.

*C. Colensoi* Hook. f., as shown by the type specimens, was based mainly on the jordanon named by Petrie *C. Banksii*. The very different jordanon on which the *C. Colensoi* of Cheeseman's *Manual* (25, p. 873) is largely based is here named *C. pseudo-Colensoi*. For a description of a swarm created by the meeting of *C. Colensoi*, *C. foetidissima*, and *C. pseudo-Colensoi* see Allan (9).

367. *C. Colensoi* × *pseudo-Colensoi*.

368. *C. crassifolia* × *rigida*. D.

369. *C. depressa* × *pseudo-cuneata*.

370. *C. depressa* × *ramulosa*.

371. *C. foetidissima* × *lucida*.

372. *C. foetidissima* × *pseudo-Colensoi*.

373. *C. foetidissima* × *pseudo-cuneata*.

374. *C. grandifolia* × *lucida*.

375. *C. grandifolia* × *robusta*.

376. *C. grandifolia* × *tenuifolia*.

377. *C. lucida* × *robusta*.

378. *C. parviflora* × *propinqua*.

379. *C. parviflora* × *ramulosa*.

380. *C. parviflora* × *rigida*.

381. *C. propinqua* × *robusta*.

For a discussion of the group see Allan (1). By crossing the two species Allan raised a diverse progeny, which matched the contents of a large wild

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swarm (3, 12). Cockayne, by sowing seeds from a supposed hybrid, procured many plants which might well have been collected from a wild swarm.

382. *C. pseudo-cuneata* × *ramulosa*.

383. *C. rhamnoides* × *rotundifolia*. D.

384. *C. rotundifolia* × *tenuicaulis*.

Certain forms are described by Carse (23) under the name × *C. gracilicaulis*.

385. *Nertera Cunninghamii* × *depressa*. D.

The two are closely allied. They occur together and apparently hybridize in the northern part of South Island.

#### CAPRIFOLIACEAE.

The single genus, *Alseuosmia*, founded by A. Cunningham, is exceedingly polymorphic. Cunningham (41) created eight species to contain the assemblage of forms he collected. Later botanists recognize four only, but except for certain isolated jordanons, nothing definite is known about the status of these 'species'. For a discussion see Allan (5). The hybrid groups listed by us must be accepted merely as a preliminary arrangement, based on herbarium material together with some knowledge of jordanons in parts of the region far distant from the centre of intense polymorphy (the northern part of North Island).

386. *Alseuosmia Banksii* × *linariifolia*.

387. *A. Banksii* × *macrophylla*.

388. *A. Banksii* × *quercifolia*.

389. *A. linariifolia* × *macrophylla*.

390. *A. linariifolia* × *quercifolia*.

#### CAMPANULACEAE.

391. *Pratia angulata* × *perpusilla*. D.

Specimens collected by Carse, and others by Zotov, give support to this group. *P. perpusilla* is rare, and the two species seldom come together. *P. angulata* is a very compound species; a number of the jordanons are in cultivation in the Wellington Open Air Museum.

*Wahlenbergia gracilis*.

This is a linneon badly needing study. The flowers range from a true blue to white, and the stature, woodiness, and form of leaves are very diverse in different forms. N. E. Brown (17) restricts the name to the type specimen from Forster's herbarium which has not been exactly matched by any subsequently collected. Brown creates his *W. Colensoi* for various specimens that had been placed under *W. gracilis*. But the situation is

much more complex than this would indicate, and the whole group should be taken up anew.

## STYLIDIACEAE.

392. *Phyllachne clavigera* × *Colensoi*. D.

## COMPOSITAE.

393. *Brachycome pinnata* × *Sinclairii*. D.

*B. pinnata* is very distinct from some forms of *B. Sinclairii*, but as Cheeseman puts it (25, p. 908) 'some varieties of that plant approach it so closely as to be almost indistinguishable'. We have little doubt that hybrids occur when the two meet, but in the absence of *pinnata*, *Sinclairii* is still extremely polymorphic and needs detailed study.

*Cassinia*.

As with so many New Zealand genera, *Cassinia* is very imperfectly known, and *C. leptophylla*, *C. Vauvilliersi*, *C. fulvida*, as at present delimited, are linneons containing probably numerous jordanons.

394. *Cassinia albida* × *fulvida* var. *montana*.

This and other *Cassinia* groups have probably greatly increased since the advent of man, through destruction of forest and through rough cultivation on montane sheep runs. Thickets of *Cassinia* are common in many localities and show strong polymorphy.

395. *C. albida* × *Vauvilliersi*.

These hybrids are confined to the north-eastern parts of South Island. Though *C. albida* is not known to occur in the south of South Island certain hybrids there found look exactly as if it were one of the parents. Forms like *C. albida* also occur in the *C. leptophylla* series.

396. *C. amoena* × *retorta* = × *C. amoenatorta* Carse.

*C. amoena* is confined to the extreme north of North Island, while *C. retorta* comes much farther south. See Carse (23).

397. *C. fulvida* var. *montana* × *Vauvilliersi*.

398. *C. retorta* × *Vauvilliersii*.

Carse collected undoubted specimens of this group, as of No. 396.

399. *Celmisia argentea* × *longifolia*.

Hybrids of this origin have been observed by Messrs. Simpson and Thomson on mountains near Dunedin, South Island. They closely mimic the group produced by No. 423.

400. *C. Armstrongii* × *coriacea*.

Did not the species *C. lanceolata* breed true, it might well be regarded as one of these hybrids; also *C. coriacea* is absent from the Longwood Range where *C. lanceolata* was first noted.

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401. *C. Armstrongii* × *Lyallii*.

We restrict the name *Lyallii* to the jordanon so called by all local botanists, though Hooker included under his species specimens of the very distinct jordanon later named *C. Petriei*.

402. *C. Armstrongii* × *Petriei*.

403. *C. Armstrongii* × *spectabilis*.

404. *C. Bonplandii* × *DuRoiizii*.

We are proposing the name *DuRoiizii* for that group of jordanons known to local botanists as *C. Sinclairii*. Hooker's *Sinclairii* was based on specimens that differ among themselves, and have not been matched in any subsequent collections.

405. *C. brevifolia* × *ramulosa*.

406. *C. brevifolia* × *Walkerii*.

407. *C. campbellensis* × *vernicaosa*.

408. *C. coriacea* × *longifolia*.

Certain forms of this cross are remarkable mimics of *C. Morgani* Cheesem., so much so that we were persuaded that *C. Morgani* belonged to this hybrid group. A study by Allan of the two localities whence *C. Morgani* was obtained showed, however, that that was a good jordanon, although epharmoning greatly according to habitat, and that the suspected parents were not present—a good example of the danger of basing taxonomic views purely on herbarium evidence!

409. *C. coriacea* × *Lyallii*.

410. *C. coriacea* × *petiolata*.

411. *C. coriacea* × *Petriei*.

412. *C. coriacea* × *spectabilis*.

× *C. Christensenii* Ckn. and *C. Bowcana* Petrie both belong to this group.

413. *C. coriacea* × *Traversii*.

× *C. Morrisonii* Ckn. belongs to this group, which contains a number of handsome forms.

414. *C. coriacea* × *verbascifolia*.

415. *C. densiflora* × *prorepens*.

416. *C. discolor* × *DuRoiizii*.

Both 'species' are very compound, and offer great difficulties in classification that can only be overcome by much field and cultural study.

417. *C. discolor* × *incana*.

418. *C. discolor* × *Walkerii*.

419. *C. DuRoiizii* × *incana*.

420. *C. Haastii* × *Hectori*.

*C. Popelwellii* Petrie probably belongs to this group.

421. *C. hieracifolia* × *oblonga*.

422. *C. longifolia* × *Monroi*.

423. *C. longifolia* × *sessiliflora*.

Many forms attributed to *C. linearis* Armst. are members of this group.

424. *C. longifolia* × *spectabilis*.

425. *C. longifolia* × *verbascifolia*.

426. *C. Lyallii* × *Petriei*.

427. *C. Lyallii* × *spectabilis*.

All the known examples of this cross are so alike, apparently being sterile  $F_1$ , that for long they were regarded as forming a 'good' species—*C. pseudo-Lyallii*. Field studies of distribution leave no doubt as to the hybridity of the forms concerned. A number of other of the *Celmisia* crosses have a comparable uniformity. How far apogamy plays a part has not been tested.

428. *C. Lyallii* × *viscosa*. D.

The two come together fairly often, but only rarely have intermediate forms been observed.

429. *C. petiolata* × *spectabilis*.

*C. lanigera* Petrie and *C. mollis* Ckn. belong to this group.

430. *C. petiolata* × *verbascifolia*.

431. *C. spectabilis* × *Traversii*.

432. *Cotula dioica* × *pulchella*.

433. *C. lanata* × *plumosa*.

434. *C. obscura* × *pulchella*.

435. *C. pectinata* × *sericea*.

436. *C. pulchella* × *Trailii*.

437. *Craspedia incana* sp. nov. ined. × *uniflora*.

The New Zealand species of *Craspedia* remain in a very confused state. The very distinct jordanon we are here calling *C. incana* is a snow-white, intensely woolly plant confined to unstable alpine or subalpine fields of stony debris. Near the margins of such screes, where the ground has become stable, forms of the linneon *C. uniflora* may be found, along with various hybrid intermediates. *C. incana* has been placed under the Tasmanian *C. alpina* Backh., but is certainly not that species, which is, however, very close to the New Zealand forms originally placed by Hooker as var. *lanata* of *C. uniflora*.

438. *Erechtites arguta* × *quadridentata*.

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439. *E. glabrescens* × *prenanthoides*. D.

On forest margins in western South Island, forms occur, in the presence of the two species, that suggest hybridism.

440. *Ewartia Sinclairii* × *Helichrysum bellidioides*. D.

The only example so far met with was described by Cockayne as *Helichrysum Fowerakeri*, who remarked that its resemblance to the above two species hinted at hybridism. So far Mr. W. Martin, who is studying the high mountain flora of the locality whence the specimens came, has not rediscovered this hybrid.

441. *Gnaphalium keriense* × *Lyllii*.

442. *G. keriense* × *subrigidum*.

The group has been discussed by Allan (11).

443. *G. luteo-album* × *purpureum*.

*G. purpureum* is an introduced species, common in North Island. A swarm of hybrids was discovered by Allan in the Volcanic Plateau Area. Cunningham (42) identifies a rust found on one of the hybrid forms as *Puccinia gnaphaliicola*, originally described from specimens on *Gnaphalium* sp. collected at Rio de Janeiro.

444. *G. Lyallii* × *trinerve*.

445. *G. MacKayi* × *Traversii*.

This group apparently occurs wherever the two species come together.

446. *G. trinerve* × *Helichrysum bellidioides*.

Examples of this intergeneric hybrid were discovered by Messrs. Simpson and Thomson near Dunedin. The hybrids grow luxuriantly and flower freely. Cockayne has also found it on the shores of Foveaux Strait. The flower heads are sometimes solitary as in the one species, or more or less numerous as in the other. The leaves also show great diversity. Several forms are in cultivation in the Wellington Open Air Museum.

447. *Helichrysum bellidioides* × *filicaule*.

448. *H. bellidioides* × *glomeratum*.

The first specimens found of this group were named *H. Purdiei*, and the species was considered purely coastal. Later Christensen, in the mountain area near Hanmer Plains, South Island, proved that the group included a number of distinct forms ranging from prostrate to lianoid, and with most diverse inflorescences. Forms have now been found in various localities. This crossing of a stout, fairly tall shrub and a slender herbaceous mat-plant was certainly unexpected.

449. *H. coralloides* × *Selago*.

This, and the next two groups, together with No. 454, are remarkably

exemplified in the mountains near the Hanmer Plains. The four species concerned hybridize in the most complicated manner. In localities where the species grow apart no such polymorphy is found.

450. *H. depressum* × *microphyllum*.

451. *H. depressum* × *Selago*.

452. *H. depressum* × ? *Raoulia tenuicaulis*.

This is represented by a single plant collected by Allan near the Ball Hut, Mount Cook. A close search revealed no more. *H. depressum* is common in the locality, and is certainly one parent. The plant has grown luxuriantly and flowers freely. The result of sowing seed is not yet available. It is possible that *Helichrysum filicaule* is the other parent, though it was not observed in the immediate neighbourhood.

453. *H. filicaule* × *glomeratum*.

Forms of this group mimic those of *H. bellidioides* × *glomeratum*. Wall (60) suggests that *H. dimorphum* Ckn. (a strong climber) is a cross between *H. filicaule* (a slender mat-plant, sometimes scandent) and *H. depressum* (a low shrub).

454. *H. microphyllum* × *Selago*.

455. *Lagenophora petiolata* × *pumila*.

456. *Leucogenes grandiceps* × *Raoulia bryoides*.

*Raoulia Gibbsii* Cheesem. belongs in part to this group, and so, too, *Helichrysum pauciflorum* Kirk. The hybrids range from small cushion-like plants to erect slightly woody ones, of which *H. pauciflorum* is a good example. The latter has never been duplicated by any wild plant, though rather similar ones have been found.

457. *L. grandiceps* × *Raoulia Goyeni*.

The known forms of this group are placed by Cheeseman (25, p. 972) under *Raoulia Loganii*. *R. Loganii* (Buch.), however, belongs to No. 459. The group under consideration is confined to Stewart Island. *Helichrysum Grahamsi* Petrie is possibly *L. grandiceps* × *H. Selago*.

458. *L. Leontopodium* × *Raoulia grandiflora*. D.

459. *Leontopodium* × *Raoulia rubra*.

*R. rubra* is confined to the Tararua Mountains, whence *Haastia Loganii* Buch. was obtained. All the forms so far collected rather closely resemble one another, so that they simulate a jordanon.

460. *Olearia albida* × *furfuracea*.

461. *O. angustifolia* × *Colensoi*.

*O. Trailii* Kirk is one form of the group, which is only known from Stewart Island. The *O. Colensoi* is not the common mountain jordanon but comes much nearer, with its large leaves, to *O. Lyallii* of the New Zealand Subantarctic Islands.

462. *O. arborescens* × *avicenniifolia*.

463. *O. arborescens* × *capillaris*.

*O. capillaris* is here used as referring to a very small-leaved divaricating shrub of North Island and the northern part of South Island. The type specimens, however, of *O. capillaris* Buch., belong either to the hybrid series or to a large-leaved epharmone. The group is represented by numerous very polymorphic swarms.

464. *O. arborescens* × *ilicifolia*.

Swarms belonging to this group discovered by Messrs. Simpson and Thomson contain a form indistinguishable from the *O. macrodonta* so common in gardens. This, again, is known to produce offspring not perfectly uniform. It may be that *O. macrodonta* is based on hybrid material. Forms, however, that would certainly be attributed to *macrodonta* occur in quantity and with uniformity of individuals in certain localities. Our present field knowledge does not enable us to decide whether this form is a jordanon or an F<sub>1</sub> hybrid.

465. *O. arborescens* × *lacunosa*.

*O. suavis* Cheesem. belongs to this group. The long narrow leaf of *A. lacunosa*, with its network of veins enclosing hollows on its under surface gives an unmistakable character to those hybrids of which it is a parent. Generally the species crossing with it are of much wider range, and these remarkable hybrids suddenly appear when its domain is reached.

466. *O. avicenniifolia* × *moschata*.

This group includes *O. Haastii* Hook. f., a form very well known in gardens in England. Forms closely resembling it have been observed in the wild state, but *O. Haastii*, as understood by New Zealand botanists, includes several other forms, and may include a true jordanon.

467. *O. avicenniaefolia* × *nummularifolia*.

468. *O. avicenniifolia* × *odorata*.

*O. Willcoxii* Petrie probably belongs here, and possibly *O. oleifolia* Kirk.

469. *O. chathamica* × *semidentata*.

On the high country to the south of Chatham Island *O. semidentata* occurs in abundance in bogs, while *O. chathamica* is chiefly in evidence on the drier ground at the margin of the sea-cliffs. It is there that the hybrids occur, one of which is Cockayne's var. *Dendyi* of *O. chathamica*. *O. semidentata* itself is extremely polymorphic and must surely consist of several jordanons and the hybrids between them. On this may depend the long flowering period of some and the different degrees of intensity of colouring in their beautiful flower-heads.



470. *O. Crosby-Smithii* × *ilicifolia*.

Only a few of these hybrids have been seen ; intermediate between the two species, which are so very different in leaf form.

471. *O. cymbifolia* × *nummularifolia*.

The species differ chiefly in that in *O. nummularifolia* the leaves are flat, in *O. cymbifolia* strongly recurved. Cockayne showed by cultivation in moist air that *O. cymbifolia* could produce flat leaves, and from this he suggested that the leaf form was merely epharmonic. A better knowledge of the distribution of the plants has clearly shown that each species when growing in the absence of the other maintains its leaf-characters.

472. *O. divaricata* × *lineata*.

The leaves of *O. divaricata* have rust-coloured tomentum, those of *O. lineata* white. Specimens belonging to this hybrid group collected by Mr. W. A. Thomson, have some leaves white, others rusty, others with flecks of both colours, all on the same plant.

*O. furfuracea*.

This is a linneon, and certain jordanons should probably receive specific rank.

473. *O. Hectori* × *virgata*.474. *O. ilicifolia* × *lacunosa*.

*O. mollis* (Kirk) Ckn. refers to one form of this group. Some of the forms mimic *O. suavis* Cheesem., which belongs to No. 465.

475. *O. ilicifolia* × *moschata*.476. *O. moschata* × *nummularifolia*.477. *O. rani* × *Sharwia paniculata*. D.

Both species are strongly compound, and in such cases hybridity is not an easy matter to prove.

478. *Raoulia apice-nigra* × *australis*.479. *R. australis* × *lutescens*.480. *R. australis* × *tenuicaulis*.481. *R. bryoides* × *eximia*.

Both belong to the 'vegetable-sheep' type of *Raoulia*, but are very distinct. Hybrid forms appear to be not uncommon. It is not yet certain whether *R. mammillaris* belongs to the hybrid group or is a third jordanon connected with the above two hybrid forms.

482. *R. bryoides* × *grandiflora*.

Forms belonging to this group have been placed under *R. Gibbsii*, and closely mimic the forms produced by No. 456.

483. *R. glabra* × *subsericea*. D.

The species so greatly resemble each other that in the absence of

genetic tests it is uncertain whether the intermediate forms are hybrids or not.

484. *R. lutescens* × *tenuicaulis*.

485. *Senecio bellidioides* × *cassinoides*.

486. *S. bellidioides* × *Monroi*. D.

*S. Christensenii* Ckn. belongs to this group.

487. *S. Bidwillii* × *elaegnifolius*.

It is interesting to note that *S. Bidwillii* occurs in two jordanons, one confined to North Island, the other (var. *viridis*) confined to the north of South Island. There are thus two distinct groups of hybrids included under the above formula.

488. *S. cassinioides* × *Haastii*.

We list this on the authority of Messrs. Simpson and Thomson, who observed an example in the Mount Cook area. *S. cassinioides* is an erect shrub up to 3 m. high, *S. Haastii* is a rosette herb. That such a wide cross is not impossible is shown by the fact that spontaneous hybrids arose in Mr. W. A. Thomson's garden between the shrub *S. Hectori* and the rosette herb *S. southlandicus* (15).

489. *S. cassinioides* × *revolutus*.

490. *S. Haastii* × *southlandicus*.

The two species are at once distinguished by their leaves, green in *S. southlandicus*, and covered with white tomentum in *S. Haastii*. It is only where the two meet that forms of intermediate nature are found.

491. *S. Lyallii* × *scorzoneroides*.

Where the narrow-leaved *S. Lyallii*, with its comparatively small yellow flower-heads, grows in company with the much broader-leaved *S. scorzoneroides*, the ray-florets of which are snow-white, there is every possible combination of leaf form and ray-colour—including lemon, cream, and salmon. Few spectacles are more beautiful in the vegetation of New Zealand than mountain bogs covered with these plants—pure white when *S. scorzoneroides* alone is present, bright yellow when *S. Lyalii*, and versicolour when the two occur together.

#### SUMMARY.

1. This list of wild hybrid groups in the New Zealand Flora is presented in the persuasion that it is of much more than local interest, as showing the conditions prevailing in a flora long isolated and now coming more and more under the influence of man.

2. The importance of the findings for all branches of botanical study, especially taxonomy, is stressed.

3. Of the 491 groups listed 396 are considered as established beyond reasonable doubt. Of these 6 are intergeneric crosses, the remainder being distributed among 45 families and 92 genera, involving 478 species, or over 20 per cent. of the flora as it is at present delimited. Were genera with only one species and extremely rare species excluded the percentage would be over 25.

4. The need for giving full weight to the phenomena of epharmony in field studies is stressed, and the desirability of further genetic study is recognized, but it is emphasized that sound field study can establish the fact of wild hybridism in the majority of cases.

5. Very many of the groups are extremely polymorphic, and show considerable fertility in the hybrid progeny.

6. In 7 cases crosses are known between exotic and indigenous species, while over 50 of the species recognized by Cheeseman (25) are now known to be based on hybrid material.

7. In certain groups characters not observed in the parents appear in the progeny, and in certain swarms (i.e. actual field occurrences of hybrid populations), especially in *Hebe* and *Leptospermum* the parents appear to be swamped by the offspring.

8. Large genera especially rich in hybrid groups are: *Acaena*, *Aciphylla*, *Asplenium*, *Carex*, *Celmisia*, *Coprosma*, *Dracophyllum*, *Epilobium*, *Gentiana*, *Hebe*, *Olearia*, *Ranunculus*, *Uncinia*.

9. Hybrids appear to be comparatively rare in the Cruciferae, and more or less sterile in *Rubus*.

10. Taking the classes (a) endemic  $\times$  endemic, (b) endemic  $\times$  wide, (c) wide  $\times$  wide: 32 per cent. of the theoretically possible groups are known in a, 5 per cent. in b, and 13 per cent. in c. (The wides form about 20 per cent. of the flora).

11. Classified according to life forms we have 178 groups of hybrids between herbs, 37 between semi-woody plants, 116 between shrubs, 27 between trees, 24 between shrubs and trees, 2 between hemi-parasites, 6 between lianes, 3 between lianes and shrubs, 3 between semi-woody plants and shrubs. Examples of especially striking groups are *Podocarpus Hallii*  $\times$  *nivalis* (tall tree  $\times$  depressed shrub), *Cordyline australis*  $\times$  *pumilio* (tall tuft-tree  $\times$  stemless tuft-plant), *Fuchsia excorticata*  $\times$  *perscandens* (spreading tree  $\times$  liane), *Hebe Astoni*  $\times$  *buxifolia* (compressed shrub  $\times$  leafy-shrub), *Helichrysum glomeratum*  $\times$  *bellidioides* (bushy shrub  $\times$  semi-woody mat-plant), *Raoulia bryoides*  $\times$  *Leucogenes grandiceps* (dense cushion shrub  $\times$  open semi-woody plant).

12. The list should further comparative studies of wild hybridism in the floras of other countries.

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# The Distribution of Potassium in Normal and Scorched Foliage.

BY

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WALLACE (27, 28, 30) has shown that an adequate supply of potash is essential for the maintenance of a favourable water balance in the foliage of a number of plants. Under the stress of potash deficiency 'leaf scorch' develops. Certain types of fruit trees and bushes, e.g. apples, gooseberries, and black and red currants are especially susceptible, whilst the foliage of certain varieties of plums develops a chlorotic appearance in addition to the typical marginal scorch (29).

Potash deficiency has been related to the development of an apical or marginal withering or dying back of the leaves in a number of plants other than fruit trees, e.g. sugar cane (Hartt (10)), wheat, corn, and buckwheat (Smith and Butler (23)), mangolds, grass, and cereals (Russell (22)), and tea (Storey and Leach (24)).

In the present investigation the distribution of potassium in scorched and unscorched foliage has been studied and, as will be seen, certain differences in the distribution of potassium in scorched and normal foliage have been demonstrated.

## *Collection of material.*

During July and August, 1932, the plants growing in the gardens of the Department of Botany, University of Bristol, were inspected and a number of species noted on which some of the foliage exhibited an apical or marginal withering or scorching. Material from this source was used in the investigation. In addition Dr. T. Swarbrick arranged for a supply of material from the Long Ashton Research Station.

Samples of scorched and unscorched leaves were collected, both being gathered from the same plant or plants except where otherwise stated. The method of division of the leaves depended on the type of scorching or withering. First, the withered portion was removed, either by rubbing between the fingers or by cutting off, and rejected. Only the living part of the leaf was used for analysis. In leaves with apical scorch or withering

the remaining portion was divided into apical and basal parts, equal by weight. In leaves with marginal scorch the division was into central and marginal regions. Thus the analyses were carried out on two regions of the leaf, one adjacent to the source of potash, e.g. the main vascular system of the plant, and the other remote from the source of potash supply.

In Table I are incorporated details of the material on which the analyses were conducted.

TABLE I.

Species.	Date of sampling.	Source.	Type of scorching or withering.
1. <i>Iris germanica</i>	26/8/32	Dept. of Botany gardens	Apical withering
2. <i>Helianthus multiflorus</i>	26/8/32	Private garden	Apical scorching
3. <i>Aralia edulis</i>	26/8/32	Dept. of Botany gardens	Marginal scorching
4. Onion	27/8/32	Dept. of Botany gardens	Apical withering
5. Pear var. William	30/8/32	Private garden	Apical blackening
6. Apple var. Lane's Prince Albert on East Malling rootstock 2	15/9/32	Long Ashton Research Station	Marginal scorching
7. Black Currant var. King's Acre Baldwin	29/9/32	„	Marginal scorching

(1) *Iris germanica*. An apical withering, confined to the older leaves had been in evidence since mid-July. Owing to the length of the leaves they were divided into three portions, e.g. base, middle, and apex.

(2) *Helianthus multiflorus*. The scorching was confined to the older leaves.

(3) *Aralia edulis*. The scorched leaves were confined to the lower branches of the shrub, which were afforded some shelter by an adjoining hedge. The leaves on the upper exposed branches were free from scorch.

The leaves of the plant are compound, and each leaflet was divided in the manner described earlier.

(4) *Onion*. The older leaves exhibited an apical withering.

(5) *Pear* var. *William*. No regularity in the distribution of the affected leaves on the tree was noticed.

(6) *Apple* var. *Lane's Prince Albert*. Material was collected from two plots, one of which had received annual dressings of a complete fertilizer, and the other, dressings of a fertilizer lacking potash. The leaves collected were taken from terminal shoots of the current year's growth and samples were taken from the basal and apical regions of the shoot. In the complete fertilizer series a greater distance separated the basal and apical regions of the shoot than in the no potash series. The amount of scorch on the four sets of material was approximately:



(a) Complete fertilizer	Base of Shoot	Very little
(b) " "	Apex " "	Nil
(c) No potash	Base " "	Every leaf scorched
(d) " "	Apex " "	Very little.

(7) *Black Currant*. Material was collected from two plots, one of which had received a complete fertilizer, and the other a fertilizer lacking potash. Unscorched leaves were collected from the complete fertilizer plot, and scorched leaves from the no potash plot. Scorching was most severe in the basal region of the shoots.

In addition to the plants from which material was collected an apical or marginal withering or scorching of some of the foliage was observed in a variety of plants. This withering was quite distinct from the yellowing which preceded the autumnal dying of the foliage.

The withering was noted in the following plants.

Date of observations, 29/8/32.

(1) *Monocotyledons*.

Species.	Family.
<i>Typha angustifolia</i> . . . . .	Typhaceae
<i>Sparganium</i> sp. . . . .	"
<i>Acorus Calamus</i> . . . . .	Araceae
<i>Iris pumila</i> . . . . .	Iridaceae
" <i>sibirica</i> . . . . .	"
" <i>xiphioides</i> . . . . .	"
" <i>stylosa</i> . . . . .	"
" <i>pseudacorus</i> . . . . .	"
<i>Allium nutans</i> . . . . .	Liliaceae
" <i>paniculatum</i> . . . . .	"
" <i>neopolitanum</i> (= <i>A. lacteum</i> ) . . . . .	"
<i>Convallaria majalis</i> . . . . .	"
<i>Asphodelus luteus</i> . . . . .	"
" <i>liburnicus</i> . . . . .	"
<i>Hemerocallis Dumortierii</i> . . . . .	"

(2) *Dicotyledons*.

Marginal scorch was observed in four plants, in addition to those from which material was collected, being accompanied by a development of bronze pigment in the leaves, especially in the regions between the main veins.

Species.	Family.
<i>Salvia coccinea</i> . . . . .	Labiatae
<i>Helianthus giganteus</i> . . . . .	Compositae
<i>Cephalaria alpina</i> . . . . .	"
<i>Tilia mississippiensis</i> . . . . .	Tiliaceae

The frequent occurrence of this apical withering amongst the monocotyledons is of interest in view of the fact that they are mainly 'sugar-leaf'

plants (9), and sugar-producing plants are recorded as having high potash requirements (22).

### Analytical data.

The analytical results are incorporated in Table II. The potash content is expressed as per cent. of dry weight, and also as per cent. of fresh weight where the fresh weight of the sample was determined.

The potash gradients in columns v and vii were obtained as follows:

$$\frac{\text{Basal (or central) conc.} - \text{Apical (or marginal) conc.}}{\text{Basal (central) conc.}}$$

Multiplication by 100 gives the gradient as a per cent. of the basal (central) concentration. Thus a positive gradient indicated a higher concentration of potash in the basal (central) region than at the apex (margin), a negative gradient indicating the reverse condition.

TABLE II.

Showing (1) dry matter content and potash content in various regions of scorched and unscorched foliage.

(2) Potash gradients in scorched and unscorched foliage.

#### 2 a. *Iris germanica*.

Material.	Region of leaf.	Dry matter %.	On D. W. basis. $K_2O$ %.	Gradient %.	On F. W. basis $K_2O$ %.	Gradient %.
Withered leaf	Base	8.9	5.81	+ 36	0.52	- 6
	Middle	11.5	5.00		0.57	
	Apex	14.8	3.72		0.55	
Normal leaf	Base	9.2	6.17	+ 31	0.57	- 4
	Middle	11.3	5.25		0.59	
	Apex	14.7	4.00		0.59	

#### 2 b. *Helianthus multiflorus*.

Scorched leaf	Base	17.8	3.79	+ 15	0.78	+ 14
	Apex	24.3	3.21		0.67	
Mature no scorch	Base	15.7	4.65	+ 20	0.69	- 6
	Apex	18.5	3.73		0.73	
Young leaf	Base	17.7	4.48	+ 25	0.65	- 20
	Apex	19.5	3.35		0.79	

#### 2 c. *Aralia edulis*.

Scorched leaf	Centre	29.4	1.57	+ 12	0.461	+ 1.0
	Edge	33.8	1.35		0.456	
Normal leaf	Centre	29.7	1.85	+ 5	0.545	- 2.5
	Edge	32.1	1.75		0.559	

#### 2 d. Onion.

Tip withered	Base	8.6	4.52	+ 24	0.396	+ 20
	Apex	9.2	3.44		0.315	
Normal	Base	6.3	4.62	+ 17	0.391	+ 16
	Apex	8.8	3.82		0.329	

2 e. Pear var. William.

Material.	Region of leaf.	Dry matter %.	On D. W. basis.		On F. W. basis.	
			K <sub>2</sub> O %.	Gradient %.	K <sub>2</sub> O %.	Gradient %.
Tip blackened	Base	—	2.62	+ 11		
	Apex	—	2.33			
Normal	Base	—	2.31	+ 9		
	Apex	—	2.09			

2 f. Apple var. Lane's Prince Albert on East Malling rootstock 2.

Complete manure series.

Base of shoot	Centre	45.5	1.56	+ 15	0.709	+ 15
	Edge	45.2	1.33		0.603	
Apex of shoot	Centre	38.0	1.61	+ 6	0.611	+ 3
	Edge	39.5	1.51		0.594	

No potash series.

Base of shoot	Centre	49.0	0.77	+ 22	0.377	+ 14
	Edge	53.8	0.60		0.323	
Apex of shoot	Centre	44.4	0.87	+ 11	0.384	+ 4
	Edge	47.8	0.77		0.370	

2 g. Black Currant var. Baldwin.

Complete fertilizer series.

No scorch	Centre	—	1.236	+ 23		
	Edge	—	0.956			

No potash series.

Scorched	Centre	—	0.823	+ 51		
	Edge	—	0.399			

D. W. = Dry weight  
F. W. = Fresh weight

From Table II it is seen that the dry matter content tends to be highest in the scorched leaves; whilst the potash content is, with the exception of the pear, lower in the scorched than the unscorched foliage.

The very high content of potash as per cent. dry matter in the iris and onion is noticeable.

Potash as per cent. of dry matter is always higher in the basal and central portion of the leaf than in the apical or marginal regions. This is so in both scorched and unscorched foliage.

With the exception of the sunflower the potash gradient is steeper in the scorched than in the normal foliage. Even with the sunflower, when the gradients of potash on a fresh weight basis are considered, the negative gradient of potash in the normal foliage is transformed into a positive gradient in the scorched leaves. Similar changes in the gradients of potash as per cent. of fresh weight are observed in the other plants wherever the differences are of any considerable magnitude.

*Discussion.*

The data suggest a relation between the potash content of the dry matter and the dry matter content of the leaves. This suggestion is confirmed by a statistical analysis of the data. Correlation Coefficients have been extracted as under.  $r$  = Correlation Coefficient between dry matter as per cent. fresh weight and potash as per cent. dry matter.

Data for iris, helianthus, onion, and apple taken together.

$$r = -0.9218 \pm 0.0275 \text{ (Standard Error).}$$

When the different sets of material are considered separately values for  $r$  are obtained as follows :

Iris	$-0.8218 \pm 0.1315$ (Standard Error)
Helianthus	$-0.6181 \pm 0.2519$
Aralia	$-0.3360 \pm 0.4412$
Onion	$-0.5500 \pm 0.3488$
Apple	$-0.7111 \pm 0.1748$

Only in the iris, sunflower, and the apple can these correlation coefficients be considered as definitely significant. The number of observations, however, is small, and it is noticeable that where the number of observations is greatest, there the correlation coefficient has the greatest significance. Bearing this in mind, the very definite trend shown by the data cannot be ignored. This, coupled with the fact that similar relations have been established by James (14) and Mann (17) does, it would seem, justify one in stating that, in so far as the data presented here are concerned, there is a definite negative correlation between the dry matter as per cent. of fresh weight and potash as per cent. of dry weight.

No relation was found to exist between the dry matter content of the leaf and the potash content expressed as per cent. of the fresh weight.

As regards the actual contents of potash in the leaf, the scorched leaves, with the exception of the pear, show a lower per cent. of potash in the dry matter than the unscorched foliage. The differences between the two types of foliage are not usually of the same order of magnitude as have been found by Wallace to exist between scorched and normal foliage. An explanation of this discrepancy is, however, available. The data of Wallace refer to extreme cases. This applies, so far as the present data are concerned, only to the black currant, and in it the difference between the content of potash in the scorched and normal foliage is of the same order as the differences between normal and scorched foliage found by Wallace.

In the present instance, the basal leaves of the shoots from the no potash series of the apples were severely scorched, and the apical leaves almost free from scorch, and one would unhesitatingly suggest potash

supply as being one of the main causal agents in bringing about the scorching of the basal leaves. Yet the potash content of the basal leaves is not very much lower than that of the apical leaves. In fact the difference is probably near the minimum likely to be found between scorched and unscorched leaves, rather than near the maximum.

By analogy a similar condition might hold for the other sets of material.

The data presented definitely establish the existence of a potash gradient on the dry weight basis, in the foliage of the plants examined, the gradient being steeper in the scorched than in the unscorched foliage.

How this difference in potash gradient between the two sets of foliage is brought about is not so clear.

Observations by Wallace (27) and Barker (2) show that scorching or withering is not apparent early in the season, but as the season advances the scorching becomes apparent, the older leaves exhibiting the symptoms first. In view of our knowledge as to the mobility of potash in the plant (André and Demoussy (1), Janssen and Bartholomew (15), Mason and Maskell (18), Gregory and Richards (8)), and concerning the variation in potash content of foliage leaves throughout the season (Gregory and Richards (8), Tucker and Tollins (25)), it seems possible to suggest that the scorching develops following the withdrawal of potash from the first formed leaves, and its translocation to the growing-point and younger leaves and possibly also in certain instances to developing fruits.

Coincident with this withdrawal of potash from the older leaves, there is an import of potash via the transpiration stream, and hence both the amount and distribution of potash in the leaf at any moment will be the resultant of these two opposing factors. When the potash supply is low or potash withdrawal considerable, then the potash content of the leaf will be seriously depleted.

The observations which have been here discussed make it permissible to suggest that the movement of potassium from the older foliage, leaves the older leaves not only with a lower potash content but also with a steeper potash gradient. Consequently the regions of the leaf remote from the main veins, e.g. the apex and edge, are left relatively poorer in potash than are the basal and central regions of the leaf. It is these regions of the leaf remote from the main veins which suffer from scorch, and, in view of the very definite relation which is known to exist between potash supply and susceptibility to scorch, the distribution of the scorched areas in the leaf would appear to be not unconnected with the relative poverty of the apices and edges of mature leaves in potash. ♀

The exact mechanism whereby scorching develops still awaits an explanation.

Summers (25) has suggested that scorch develops following a rapid

breakdown of water supply to the leaf. He concluded also that the development of the brown colour is due to enzymatic action upon chromogens formed during the drying out of the leaf.

Mann (17) found that not only do leaves from potash-deficient apple trees show a low water content, but they may also possess poor resistance to water loss.

More recently Wallace (28) has suggested that scorch develops under conditions favouring a disturbance of the water supply within the leaf, and is not so much related to the supply of water to the leaf or to transpiration.

Penston (21) has shown that withdrawal of potash from the leaf of the potato which occurs whilst the leaf is still green affects first the mesophyll and palisade cells in which a considerable proportion of the potash of the leaf is normally located, and suggests that potassium withdrawal may be a causal factor in the yellowing of the leaf. The regions of the leaf most affected by this withdrawal are the regions remote from the vascular bundles.

That scorch is a phenomenon distinct from wilting has been pointed out (Barker 2). An observation made during the summer of 1932 bears this out.

A weeping ash-tree in the gardens of the Department of Botany exhibited severe wilting during the extremely hot weather in August 1932 when shade temperatures of over 90° F. were registered. The sheltered leaves of the tree were those most affected. The wilting was so severe and so prolonged as to result in the death of much of the affected foliage. Always, however, a leaf either died and shrivelled completely or recovered. No instance of a leaflet showing an apical or marginal scorching or shrivelling was recorded.

These observations, however, throw no light on the mechanism of the development of the scorch. That the maintenance of the favourable water balance is in part at least dependent upon an adequate supply of potash would seem to be evident.

The nature of the relation between water balance and potash supply is obscure. De Vries (1884) (6) suggested that the power of vacuoles to absorb water was due in part to dissolved potassium salts. Copeland (1897) (4) put forward the view that a relation existed between turgor and nutrient salts, especially potassium. Weevers (31) believed that potassium functioned in the regulating of the turgidity of cells. James (14) has pointed out that the potassium present is, in many cases, capable of exerting an osmotic pressure representing a considerable proportion of the maximum osmotic pressure recorded by Dixon (7), and that a higher concentration of potash will result in a greater ability on the part of the cell concerned to maintain its turgidity. Especially will this be the case if, as has been suggested by Kotyschew and Eliasberg (16), all the potash of the cell exists

in the ionic form. Further, as potash salts in many plants seem to be the predominant soluble salts (1), the effect of other minerals in this respect will be small compared to that of potassium.

If withdrawal of potash from the leaves of other plants follows the same course as in the potato, then the assimilating areas remote from the main veins will be the regions of the leaf most affected by this withdrawal of potash. A withdrawal of potash resulting in a concentration of potash in the cells concerned below a certain minimum must result in a serious diminution of photosynthetic activity (Briggs (3), Gregory and Richards (8), Janssen and Bartholomew (15), Nobbe (19)). The cells thus affected would be expected to show a greater loss of osmotic pressure than would be accounted for by the potash withdrawal. Any loss of osmotic pressure in these cells due to diminution in the rate of carbohydrate production would of course only be apparent when the potash content had been reduced to such an extent that potash supply became the factor 'limiting' assimilation. Hence in leaves with a high initial potash content, or in which the import of potash was high, considerable withdrawal of potash might be possible before the stage of potash starvation was reached.

Apart from any such direct effect potash salts will have an indirect effect. Diminution in water content following potash withdrawal may lead to reduction in photosynthetic activity. Such an effect would be superimposed on the direct effect of potash starvation. A serious diminution in the ability to produce osmotically active substance might, it is suggested, result in a failure of the osmotic mechanism of the cells concerned and be reflected in a drying out of these cells, i.e. in the development of leaf-scorch. Clearly, as long as the basal portion of the leaf retains the ability to maintain a favourable water balance wilting will not occur.

It must be pointed out that the potash gradients which have been discussed are to be considered as dynamic and not static gradients. Withdrawal and import of potash resulting in a lower concentration of potash in the leaf, and a steepening of the potash gradient, are two continuous and opposing processes.

It is not suggested that such an hypothesis is capable of explaining the mechanism of all types of withering. Withering which is not connected with mineral deficiencies certainly does occur. (Hansen (11), Hansen-clever (12), Oliver (20), Yapp (32, 33).)

The writer wishes to express his indebtedness to Dr. M. Skene for his helpful criticism.

#### SUMMARY.

(1) Scorching and withering in the foliage of a number of plants is described.

(2) Data relative to the chemical composition of scorched and unscorched foliage are presented.

(3) A negative correlation between dry matter content of the leaf and potash content of the dry matter is found.

(4) It is shown that scorched leaves compared with unscorched foliage show (a) a lower content of potash in the dry matter, (b) a steeper potash gradient.

(5) A possible explanation of the mechanism of the development of leaf scorch is described.

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# Studies in the Genera *Cytosporina*, *Phomopsis*, and *Diaporthe*.<sup>1</sup>

## V. Analysis of Certain Chemical Factors Influencing Fungal Growth in the Apple.

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and Technology, London.)*

With twenty-six Figures in the Text.

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### I. INTRODUCTION.

IN the third and fourth papers of this series (3, 4) Das Gupta dealt exhaustively with the subject of the attacking power of strains, and found that certain strains differ significantly in attacking power. It seemed desirable to pursue this matter further by means of an analysis of factors influencing growth of strains, in order to find out, if possible, what factors are responsible for the differences observed. It was also thought very probable that the analysis would shed considerable light on changes of rate of invasion observed by Horne (6) who has worked mainly with

<sup>1</sup> Thesis approved for the Degree of Doctor of Philosophy in the University of London.

*Fusarium*, *Diaporthe*, *Cytosporina*, and *Phomopsis*.

Horne (7) and Horne and Gregory (10) have expressed the opinion that changes in rate of invasion are related to chemical composition of apple fruit. Thus, acid is regarded as an important factor, and the deduction is based on such evidence as that derived from inoculation experiments with apples of known acidity or pH, experiments on growth of fungi in apple extracts of known acidity or pH, and so on. Changes in sugar content are also stated to affect fungal invasion. Horne (7) has made it perfectly clear that exact information cannot be obtained by the methods referred to above because the chemical constituents are simultaneously undergoing change with age of fruit. Moreover, apple extracts when sterilized under pressure undergo a considerable hydrolysis of disaccharides and more complex carbohydrate materials, so that a much larger supply of reducing sugars is available to the fungus than under normal conditions. It is therefore impossible to state whether rate of invasion has been affected by acid, sugar, or some other factor in any particular case.

By adopting cultural methods it is possible to study the effect of varying concentration of one factor at a time, and find out exactly in what way altered concentration affects growth. The work has been mainly confined to studying the effect of two variable factors, acid and glucose. It was deemed inadvisable to introduce a third variable, such as nitrogen, because every additional variable involves greatly augmented experimental work. Instead, experiments have been made with sucrose or fructose substituted for glucose, and with the three sugars used in varied proportions instead of glucose.

The results presented in this investigation taken in conjunction with certain results recently obtained from a study of induced changes in resistance by Horne and Seth (9) show conclusively that changes in rate of invasion may be conditioned by acid or sugar or both factors. Differences in attacking power may also be explained to a great extent in terms of growth response to concentration of acid or sugar.

## II. MATERIAL.

A list of the strains used in this investigation is given below:

- |  |  |
|--|--|
| 1. <i>Cytosporina ludibunda</i> , strain CE  | } Saltants described by Dr. S. N. Das Gupta (3).                   |
| 2. " " strain CA <sub>4</sub>  |  |
| 3. " " strain CC <sub>2</sub>  |  |
| 4. <i>Phomopsis coneglanensis</i> Trav., obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland. | } Isolated from Citrus fruits, Low Temperature Station, Cambridge. |
| 5. <i>Phomopsis citri</i> , strain Jaffa 1   |  |
| 6. " " strain Jaffa 18   |  |
| 7. " " strain Brazil 20  |  |
| 8. <i>Phomopsis vexans</i> (Sacc. and Syd.) Harter. Baarn, Holland.  |  |

9. *Diaporthe perniciosa*, strain DHF. Isolated from natural spots of Bramley's Seedling apple fruit by Dr. A. S. Horne in 1927.
10. *Diaporthe arctii* (Lasch) Nit. Baarn, Holland.
11. *Diaporthe celastrina* Ell. et Barth.
12.     "     *acerina* Pk.
13.     "     *Beckhousii* Nit.
14.     "     *strumella* (Fr.) Fck.

} Washington

The author is greatly indebted to Mr. Tomkins for the strains of *Phomopsis citri* sent from Cambridge and to Miss Jenkins for the species of *Diaporthe* received from Washington.

# METHOD.

The composition of the medium used as a standard is as follows :

Asparagin	0.2	gram.
MgSO <sub>4</sub> , 7H <sub>2</sub> O	0.075	"
K <sub>3</sub> PO <sub>4</sub>	0.125	"
Glucose	1.80	"
Agar	2.0	"
Water	100	c.c.

In preparing sugar series, the glucose in the standard medium was replaced by the kind and concentration of sugar required. For acid series malic acid was employed and several concentrations were used. The concentrations of sugars and acid chosen were, as a rule, in geometrical progression, and for the sake of convenience in the text these concentrations have been represented by the first letter of the sugar or acid employed. Thus 9 G represents 9 per cent. glucose, 13.5 S represents 13.5 per cent. sucrose, and 0.25 A represents 0.25 per cent. malic acid, and so on.

In preparing acid series, solutions of malic acid were always sterilized separately and added to the standard medium when the latter had cooled down sufficiently (to 55° C. approximately). For the glucose series the medium was sterilized at 10 lb. pressure for 15 minutes in the autoclave, but wherever fructose or sucrose was used the medium was sterilized by steaming for 30 minutes on three consecutive days.

For all the different series, sets of plates, in triplicate, were prepared and the medium poured to reach the same depth in every plate, that is 0.75 cm. The plates were inoculated with a given fungal strain and kept at 20° C. Cultures of strains used for the experimental work, the purity of which was ensured at the start by using mono-hyphal transfers from stock cultures, were kept in tubes containing standard medium. In earlier experiments such tubes were used for the purpose of inoculating plates. As time went on, it was discovered that whenever acid or high concentrations of sugars were used the replicates were not uniform. This difficulty was overcome by adopting the following method: A plate containing

standard medium was inoculated at the centre with a given fungal strain, using an inoculum taken from a tube culture, and kept at 20° C. for a week. The plate was then used for inoculating series. The inocula, consisting of very small fragments of mycelium with the least possible medium adhering to them, were taken from a narrow zone situated about 1 cm. inwards from the margin of the culture, thus ensuring that all the inocula were of the same age. This method gave very satisfactory results.

The estimates of radial spread recorded in this paper are, as a rule, based on measurements taken along two diameters at right angles to one another. In cases where the margin of the culture proved somewhat irregular additional measurements were made. Measurements were made at regular intervals of time until the ninth day, and in the case of slow growing strains additional measurements were made on the eleventh or fifteenth day. For comparative purposes the records obtained on the ninth day have been selected for the simple reason that fast growing strains reach the margin of the plate in about ten or eleven days. The method of linear measurements has been adopted in this investigation because it is analogous to the method used for estimating the progress of fungal invasion in the apple fruit (10).

### III. GENERAL OBSERVATIONS.

The general morphological characters shown by different strains in standard medium cultures are given below in Table I.

TABLE I.

*Characters of Strains as Shown in Standard Medium Plate Cultures.*

Strain.	Colour of mycelium.	Nature of mycelium.	Zonation.	Colour of substratum.
<i>Phomopsis coneglans</i>	White to grey	Thick felt	Wide	Dark brown
<i>Cytosporina ludibunda</i> , CE	Ash	do.	do.	Grey
<i>Diaporthe perniciosa</i> , DHF	White, silky	Thick felt with dense tufts of aerial mycelium	do.	White
<i>Cytosporina ludibunda</i> , CA <sub>4</sub>	Black	Thin felt	Absent	Black
<i>Cytosporina ludibunda</i> , CC <sub>2</sub>	Pale orange or yellow	Thin, rough felt	do.	Brown
<i>Diaporthe arctii</i>	White	Thin, fluffy felt	do.	White
<i>Phomopsis vexans</i>	Light brown	Thin felt	do.	Brown
<i>Phomopsis citri</i> , Brazil 20	White	Thick felt	Irregular	White
<i>Phomopsis citri</i> , Jaffa 1 and 18	do.	do.	Wide	do.

The growth characters recorded above vary, and in some cases considerably, with altered level of acid or sugar. With increasing sugar the mycelium is reduced in bulk until at the highest concentrations used growth is thin and flat and the cultures show little colour. With increasing

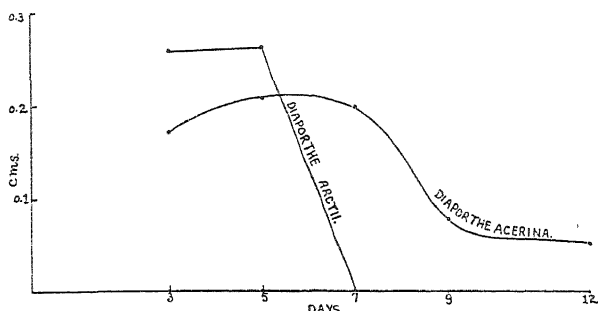


FIG. 1. Graph showing radial spread of staling strains in relation to age of culture.

acid, especially at levels beyond 0.85 A, the mycelial characters are mainly determined by the amount of sugar present in the medium: with low sugar (< 6 G, &c.) growth is flat, but as the concentration increases there is a tendency to form more and more strands or tufts of aerial mycelium.

As a rule the strains under investigation produced fairly regular growths with circular outlines in plate culture, and little difficulty was experienced in making linear measurements. Growth was less regular in media containing relatively high concentrations of acid. Correspondence between replicates was very close, except in the case of acid series containing fructose as a constituent of the carbohydrate ingredient. The replicates varied in rate of radial spread: in some plates the rate was fairly uniform; in others it slowed down, and in others growth ceased entirely after a time. In the case of *Cytosporina ludibunda*, CE and *Phomopsis coneglanensis*, this lack of uniformity was always observed in media where the level of fructose was relatively high (exceeding 8 F) even when the inocula used were of equal age.

Sectoring was occasionally observed in acid-fructose series, in the case of *C. ludibunda*, CE, and a strain differing in morphological characters from the parent was isolated from the sectors.

The strains investigated with two exceptions (*Diaporthe arctii* and *D. acerina*) proved to be of the non-staling type. The staling strains showed strong staling in all the acid series where a relatively high level of sugar was maintained. The staling character as developed in a medium containing 13.5 G is shown by the graph given in Fig. 1, where length of radius of spread is plotted against time.

In order to discover whether the strains under investigation are capable of bringing about any changes in the initial pH of the medium as

a result of metabolic activity, two strains, *C. ludibunda*, CE and CA<sub>4</sub>, fast and slow growing respectively, were grown on a medium containing three sugars (glucose, fructose, and sucrose) in different proportions (1.7, 8.6, and 2 per cent. respectively). The initial pH of the medium was 5.8 and the

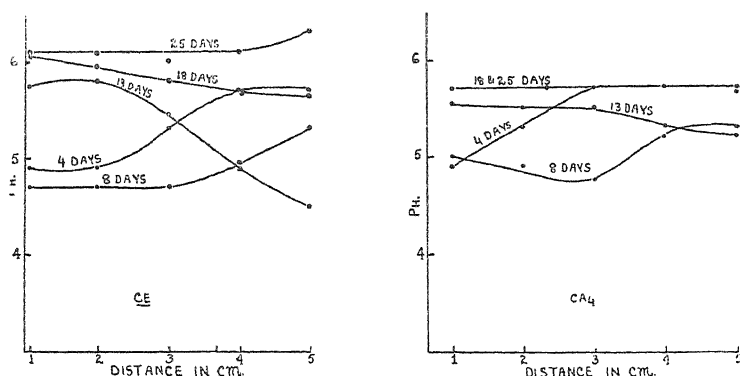


FIG. 2. Graph showing changes in pH with increasing age of culture. CE = *Cytosporina ludibunda*, CE. CA<sub>4</sub> = *Cytosporina ludibunda*, CA<sub>4</sub>.

changes in pH in different parts of the plates were estimated periodically. The changes in pH of the medium associated with the growth of these strains are shown in Fig. 2, where hydrogen-ion concentration is plotted against distance in centimetres from the inoculum.

The results obtained indicate that both strains are capable of changing the initial pH of the substratum. During the first few days of the growth period the hydrogen-ion concentration increases, but later falls and the media become alkaline. The effect observed is greater in the case of the fast growing strain, *C. ludibunda*, CE. In the case of both strains a change in pH was observed in the medium immediately in advance of the growing margin of the fungus. This experiment was repeated, using the same medium with 1 per cent. malic acid added. The initial pH of the medium was 4.3. In this case the hydrogen-ion concentration was maintained for about a week and then commenced to fall. Determinations of pH made after twenty days from an average sample of medium yielded the following result: *C. ludibunda*, CE, pH 7.0; *C. ludibunda*, CA<sub>4</sub>, pH 5.1. These results indicate that the medium tends to become alkaline. Similar results have been obtained by Culpepper (1) and others. Culpepper found that the acidity of apples attacked by *Sphaeropsis malorum* falls from an initial acidity of 0.92 per cent. to 0.30 per cent. It should be noted that the change in pH of the medium in advance of growth is very slight, therefore the actual acidity in this region would not differ very greatly from the initial values of concentrations of acid employed in this investigation.



## IV. MALIC ACID AND GLUCOSE.

(a) *Cytosporina ludibunda*. Strain CE.

The general effect of varied acid and glucose was first ascertained by using 6 concentrations of acid (0.025, 0.05, 0.1, 0.21, 0.42, and 0.85 A) and 7 concentrations of glucose (1.8, 2.7, 4.0, 6.0, 9.0, 13.5, and 17 G), making

TABLE II.

*Cytosporina ludibunda*, CE. *Length of Radius in Relation to Varying Concentrations of Glucose and Malic Acid.*

		Glucose in gram. per 100 c.c.						
		1.8.	2.7.	4.0.	6.0.	9.0.	13.5.	17.0.
Acid in gram. per 100 c.c.	0.0257	29.5	29.5	27.0	23.5	22.0	18.0	15.5
		29.5	29.5	27.0	24.0	21.0	18.0	15.5
		29.5	30.0	27.0	24.5	22.0	18.0	15.5
	0.0536	26.5	26.5	24.5	21.5	18.0	15.0	13.0
		27.5	27.0	24.5	23.0	18.0	15.5	13.0
		29.0	30.0	25.5	23.0	18.0	15.5	13.0
	0.1072	22.0	21.5	20.5	18.0	17.0	14.0	13.0
		23.0	22.0	20.5	18.5	18.0	14.0	13.0
		23.0	22.5	20.5	19.0	18.0	14.0	14.0
	0.2144	19.0	18.0	17.5	17.0	16.0	13.0	12.5
		20.0	18.0	18.0	17.0	16.0	13.0	12.5
		20.0	18.5	17.7	17.0	16.0	13.0	12.5
	0.4228	15.0	15.5	15.5	16.0	17.0	16.0	14.0
		15.5	15.5	16.5	16.5	17.0	16.5	14.0
		15.5	15.5	17.0	17.0	17.0	17.0	14.5
	0.8576	11.0	12.0	12.0	13.0	13.0	12.0	11.0
		10.5	12.5	12.0	13.0	15.0	13.0	13.0
		11.0	13.0	12.0	13.0	15.5	12.0	13.0
	1.2000	5.5	6.0	6.0	5.5	6.0	9.2	9.2
		6.0	6.5	6.0	6.0	6.0	9.2	9.0
		5.5	6.5	6.5	6.5	5.5	9.2	9.0
	1.3700	3.5	4.0	5.0	2.5	2.5	4.7	4.2
		3.5	4.0	3.5	3.0	3.5	4.2	4.2
		4.5	4.0	4.2	3.5	4.0	4.5	4.0
	1.5400	2.5	3.0	3.0	3.0	1.5	1.5	3.0
		3.0	3.5	3.0	3.0	No growth	2.5	4.7
		3.0	3.5	3.0	3.0	1.5	No growth	No growth
	1.7000	2.5	2.5	3.0	2.0	1.0	0.5	1.5
		2.5	2.5	2.5	1.5	1.0	No growth	No growth
		2.5	2.0	2.5	1.5	No growth	No growth	No growth

42 combinations in all and involving 126 plate cultures. The entire experiment was repeated twice. Graphs showing the relation between radial spread in nine days, expressed in terms of length of radius, and concentration were then made. Some of the curves obtained were of a peculiar

nature and necessitated more detailed work both within and beyond the range of acid concentration first employed, and involving 70 combinations of acid and glucose (210 plate cultures). The full data obtained are given

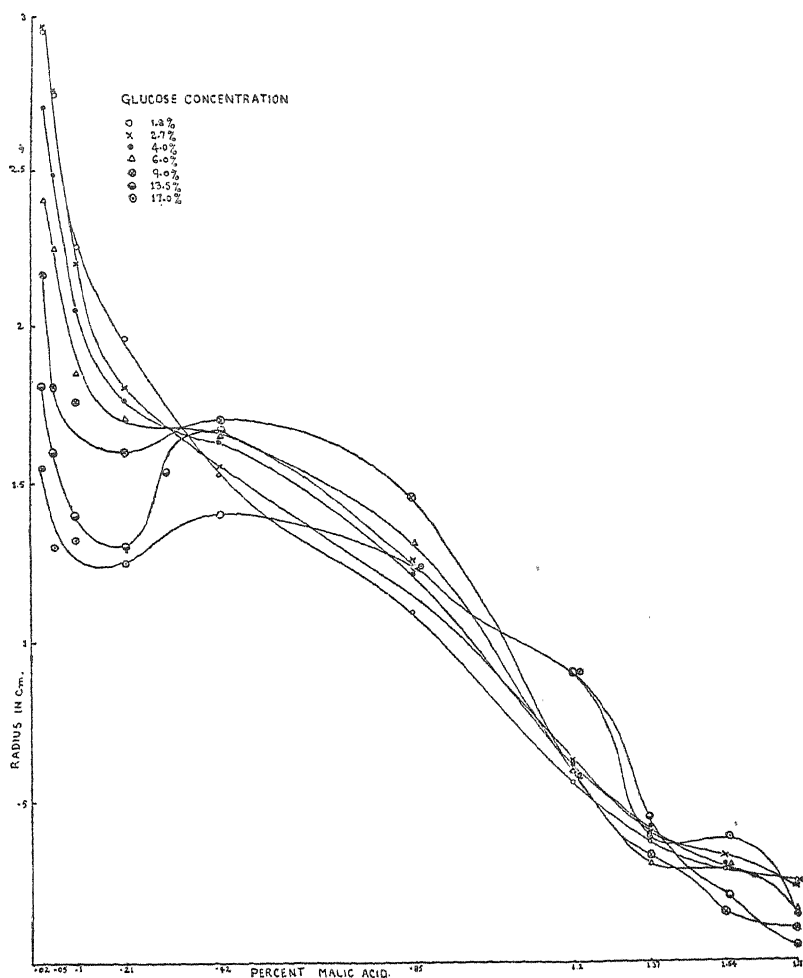


FIG. 3. Graph showing radial spread in relation to acid concentration for seven different concentrations of glucose. *Cytosporina ludibunda*, C.E.

in Table II, from which the degree of correspondence shown by replicates can be ascertained.

The data used for the graphs are in nearly every case those of mean measurements obtained for three cultures. In Fig. 3 length of radius is plotted against acid concentration for each of the seven members of the glucose series. The curves thus obtained reveal the following interesting features.

1. The curves are of characteristic form.
2. The form of the curve varies with concentration of glucose.
3. All the curves, with the exception of that given for 17 G, tend to

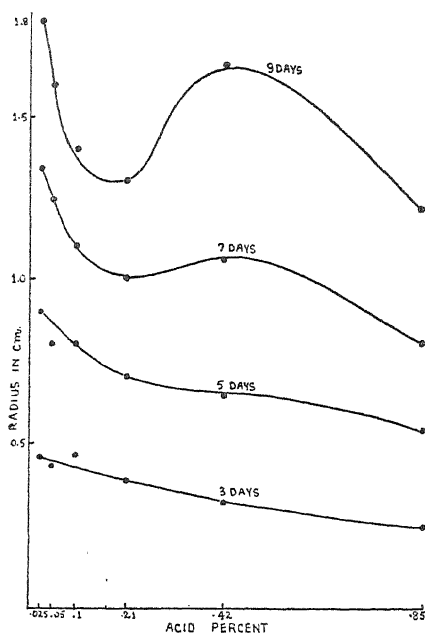


FIG. 4. Graph showing effect of age of culture on development of the second maximum. *Cytosporina ludibunda*, CE.

intersect at a point in the neighbourhood of 0.35 A, indicating that at this concentration radial spread is unaffected by varying glucose.

4. With lower concentrations of glucose (1.8, 2.7, 4 and 6 G) rate of spread falls with increasing acid.

5. With higher concentrations of glucose (9, 13.5, and 17 G) the rate falls with increasing acid until a certain critical concentration is reached and then the rate rises with increasing acid to a second maximum from which it falls again until growth ceases. The position of the second maximum has been carefully verified by exploring in greater detail the range of acid falling between 0.1 A and 0.85 A.

6. The tendency towards a second maximum is found to be most pronounced at 13.5 G.

The effect of time on development of the second maximum is shown in Fig. 4 where length of radius observed on the third, fifth, seventh, and ninth days, respectively, is plotted against acid concentration.

It is seen that no marked tendency towards a second maximum is shown on the third and fifth days. The second maximum was first observed on the seventh day, and was very prominent on the ninth day.

In Fig. 5 the values given in Table II will be found plotted against glucose concentration for the first seven members of the acid series.

These curves are also of characteristic form, and in this case the form

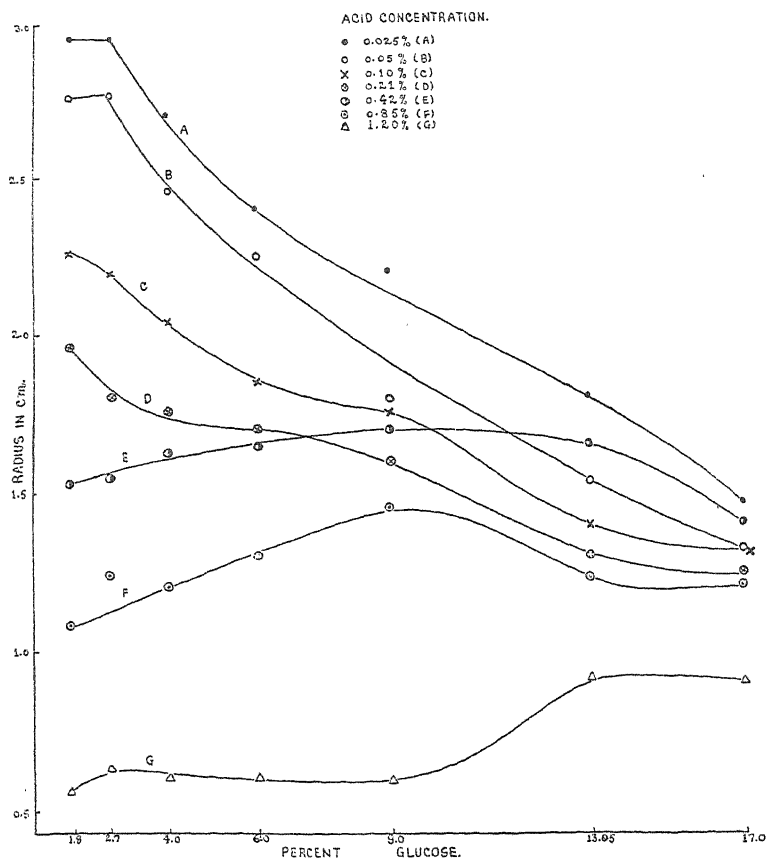


FIG. 5. Graph showing radial spread in relation to glucose concentration for seven different concentrations of acid. *Cytosporina ludibunda*, CE.

varies with acid concentration. The curves are more irregularly spaced than those given in Fig. 3 for various glucose concentrations, indicating that increasing acid exercises, on the whole, a greater retarding effect on growth. The main effects of increasing glucose are as follows:—Acid 0.025 and 0.05; the rate of radial spread falls with increasing glucose but the rate of fall gradually diminishes. Acid 0.10 and 0.21; the rate falls with increasing glucose but the curve follows a sinuous course.

Acid 0.42 and 0.85; the rate rises slightly with increasing glucose until a maximum concentration is reached and then the rate falls.

Acid 1.2; the rate remains practically unchanged up to 9 G, it then

risers until a certain concentration is reached and then the rate presumably falls.

Unlike the series of curves given in Fig. 3, these curves do not tend to intersect at one point, neither do they show any marked tendency towards development of a second maximum. The intersection of the curve E (0.42 A) with curves B, C, and D (0.05, 0.10 and 0.21 A) is due to the fact that near the point of intersection radial spread is little affected by varying glucose.

A comparison of curves constructed from data obtained on the fourth, seventh, and ninth day, respectively, shows that no very marked change in form and orientation has taken place, except in the case of the curve for 0.42 A. On the fourth day this curve does not intersect with any of the neighbouring curves, on the seventh day it intersects the curves C (0.05 A) and D (0.10 A), and on the ninth day it intersects B, C, and D as shown in Fig. 5.

When the data given in Table II are plotted using logarithmic values of glucose concentration it is found that the curve for the lowest acid (0.025 A) is approximately linear, and therefore nearly exponential in character. As the concentration of acid increases the curves depart more and more from the exponential type.

The logarithms of the values given in Table II and used in Fig. 3 have been plotted against logarithms of values of glucose concentration. The curves thus obtained are presented in Fig. 6, CE.

It is seen that the curves are of somewhat similar form. Starting from left to right in the Figure they follow a downward course and in several cases the course is nearly straight from the neighbourhood of 0.21 A onwards. Between 0.21 A and 0.85 A the course followed varies slightly with concentration of glucose, curves for high glucose bend upwards and arch over, on the other hand, those for low glucose continue their downward course. The direction of all the curves changes abruptly at about 0.85 A, the curves falling very steeply towards the base line.

Although systems of curves of this nature do not appear to have been recorded previously by pathologists, analogous systems have been described by workers in other fields of scientific inquiry. Brief reference to this subject will be made in the discussion.

#### (b) *Phomopsis coneglanensis*.

Exactly the same procedure was adopted as in the case of *C. ludi-bunda*, CE. Seventy different combinations of acid and glucose were used, and the whole experiment was carried out on two separate occasions. The full data are given in Table III.

The curves for members of the glucose series, obtained by plotting

length of radius against acid concentration are given in Fig. 7. For the sake of clearness the curves for 6 G and 17 G are omitted since the curve for 17 G resembles that obtained for 13.5 G, and the curve for 6 G differs very little from that represented by 4 G.

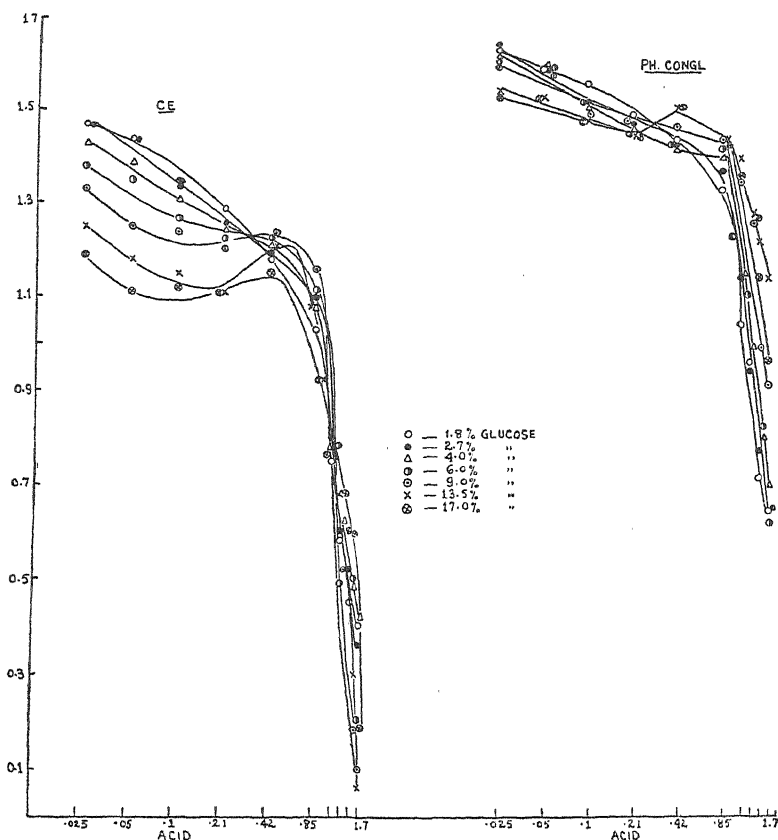


FIG. 6. Relationships shown in Figs. 3 and 7 represented by logarithmic curves. CE = *Cytosporina ludibunda*, CE. PH. CONGL. = *Phomopsis coneglanensis*.

It will be seen from Fig. 7 that this strain does not differ to any great extent from *C. ludibunda*, CE, in general form of the curves presented. The special points to be noted in connexion with this strain are as follows:

1. The point at which most of the curves tend to intersect lies between 0.2 A and 0.3 A.

2. The curve for 9 G does not show a second maximum.

3. In the case of 13.5 G the second maximum is found at a much higher concentration of acid than that observed for *C. ludibunda*, CE.

With regard to the effect of time on the development of the second maximum, development was found to take place earlier than in the case of

*C. ludibunda*, CE. In this case the second maximum was first observed on the fifth day.

TABLE III.

*Phomopsis coneglanensis*. Length of Radius in Relation to Varying Concentrations of Glucose and Malic Acid.

		Glucose in grm. per 100 c.c.						
		1·8.	2·7.	4·0.	6·0.	9·0.	13·5.	17·0.
Acid in grm. per 100 c.c.	0·025	44·0	44·5	43·0	41·5	40·0	35·5	34·5
		44·0	44·5	43·0	42·0	41·0	36·0	34·5
		44·0	45·0	43·0	43·0	43·0	35·0	35·0
	0·05	39·0	39·0	40·5	39·0	40·0	35·0	34·5
		40·0	39·5	40·5	40·0	40·0	35·0	34·5
		40·5	41·5	40·5	40·5	40·0	35·0	34·5
	0·10	37·0	33·0	34·5	34·0	33·0	31·0	29·0
		37·0	35·0	33·5	34·0	32·5	31·0	31·0
		37·0	35·0	34·5	34·0	32·5	31·0	31·0
	0·21	31·5	30·0	29·5	28·5	29·0	29·0	29·0
		32·0	30·0	29·5	30·0	30·5	29·0	29·0
		31·5	30·5	29·5	29·0	31·5	29·5	29·0
	0·42	27·5	28·0	28·0	27·0	28·0	32·0	33·5
		27·5	28·0	27·5	27·5	30·0	34·0	34·0
		29·0	28·5	28·0	27·5	30·0	33·0	34·0
	0·85	22·0	24·0	24·5	26·5	28·0	29·5	25·0
		22·0	24·0	25·5	27·0	28·5	29·5	28·0
		22·0	24·5	26·5	27·5	28·5	29·5	28·0
	1·20	10·5	12·0	13·0	16·5	22·5	23·0	21·0
		11·0	13·0	12·0	17·0	23·0	26·5	24·0
		12·5	12·0	12·0	19·0	24·0	27·0	24·5
	1·37	7·0	9·0	9·5	10·0	18·0	18·0	17·5
		8·0	9·0	10·5	10·0	19·0	20·5	17·5
		8·0	9·0	9·5	11·0	19·0	20·5	18·0
	1·54	5·5	5·5	6·0	6·5	9·0	18·0	13·0
		5·0	6·0	6·5	—	10·5	18·5	13·0
		5·5	6·5	6·5	7·0	10·5	16·0	16·0
	1·70	4·5	4·0	4·5	5·0	8·5	14·0	8·5
		4·0	4·5	5·0	3·0	8·0	14·0	9·0
		5·0	5·0	5·5	5·5	8·5	14·0	10·5

Logarithms of the values given in Table III have been plotted against logarithmic abscissae in Fig. 6 (PH. CONGL.): it will be seen that the system of curves obtained resembles closely that presented for *C. ludibunda*, CE in the same figure.

In Fig. 8 radial spread is plotted against glucose concentration for six members of the acid series. The sets of curves thus obtained show that the fall in rate of spread with increasing glucose as observed at low levels of acid is less marked than that recorded for *C. ludibunda*, CE.

length of radius against acid concentration are given in Fig. 7. For the sake of clearness the curves for 6 G and 17 G are omitted since the curve for 17 G resembles that obtained for 13.5 G, and the curve for 6 G differs very little from that represented by 4 G.

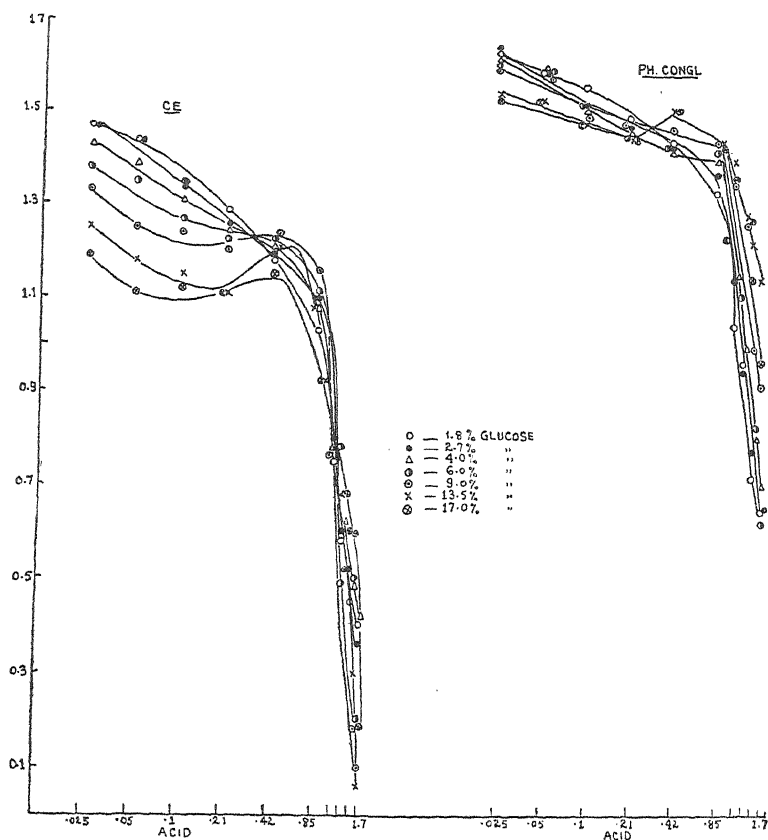


FIG. 6. Relationships shown in Figs. 3 and 7 represented by logarithmic curves. CE = *Cytosporina ludibunda*, CE. PH. CONGL. = *Phomopsis conglanensis*.

It will be seen from Fig. 7 that this strain does not differ to any great extent from *C. ludibunda*, CE, in general form of the curves presented. The special points to be noted in connexion with this strain are as follows:

1. The point at which most of the curves tend to intersect lies between 0.2 A and 0.3 A.

2. The curve for 9 G does not show a second maximum.

3. In the case of 13.5 G the second maximum is found at a much higher concentration of acid than that observed for *C. ludibunda*, CE.

With regard to the effect of time on the development of the second maximum, development was found to take place earlier than in the case of



*C. ludibunda*, CE. In this case the second maximum was first observed on the fifth day.

TABLE III.

*Phomopsis coneglanensis*. Length of Radius in Relation to Varying Concentrations of Glucose and Malic Acid.

		Glucose in grm. per 100 c.c.						
		1.8.	2.7.	4.0.	6.0.	9.0.	13.5.	17.0.
Acid in grm. per 100 c.c.	0.025	44.0	44.5	43.0	41.5	40.0	35.5	34.5
		44.0	44.5	43.0	42.0	41.0	36.0	34.5
		44.0	45.0	43.0	43.0	43.0	35.0	35.0
	0.05	39.0	39.0	40.5	39.0	40.0	35.0	34.5
		40.0	39.5	40.5	40.0	40.0	35.0	34.5
		40.5	41.5	40.5	40.5	40.0	35.0	34.5
	0.10	37.0	33.0	34.5	34.0	33.0	31.0	29.0
		37.0	35.0	33.5	34.0	32.5	31.0	31.0
		37.0	35.0	34.5	34.0	32.5	31.0	31.0
	0.21	31.5	30.0	29.5	28.5	29.0	29.0	29.0
		32.0	30.0	29.5	30.0	30.5	29.0	29.0
		31.5	30.5	29.5	29.0	31.5	29.5	29.0
	0.42	27.5	28.0	28.0	27.0	28.0	32.0	33.5
		27.5	28.0	27.5	27.5	30.0	34.0	34.0
		29.0	28.5	28.0	27.5	30.0	33.0	34.0
	0.85	22.0	24.0	24.5	26.5	28.0	29.5	25.0
		22.0	24.0	25.5	27.0	28.5	29.5	28.0
		22.0	24.5	26.5	27.5	28.5	29.5	28.0
	1.20	10.5	12.0	13.0	16.5	22.5	23.0	21.0
		11.0	13.0	12.0	17.0	23.0	26.5	24.0
		12.5	12.0	12.0	19.0	24.0	27.0	24.5
	1.37	7.0	9.0	9.5	10.0	18.0	18.0	17.5
		8.0	9.0	10.5	10.0	19.0	20.5	17.5
		8.0	9.0	9.5	11.0	19.0	20.5	18.0
	1.54	5.5	5.5	6.0	6.5	9.0	18.0	13.0
		5.0	6.0	6.5	—	10.5	18.5	13.0
		5.5	6.5	6.5	7.0	10.5	16.0	16.0
	1.70	4.5	4.0	4.5	5.0	8.5	14.0	8.5
		4.0	4.5	5.0	3.0	8.0	14.0	9.0
		5.0	5.0	5.5	5.5	8.5	14.0	10.5

Logarithms of the values given in Table III have been plotted against logarithmic abscissae in Fig. 6 (PH. CONGL.): it will be seen that the system of curves obtained resembles closely that presented for *C. ludibunda*, CE in the same figure.

In Fig. 8 radial spread is plotted against glucose concentration for six members of the acid series. The sets of curves thus obtained show that the fall in rate of spread with increasing glucose as observed at low levels of acid is less marked than that recorded for *C. ludibunda*, CE.

The curve for 0.42 A is at first nearly horizontal and then rises from 6 G with further increase in glucose, intersecting only two curves (0.1 A and 0.21 A).

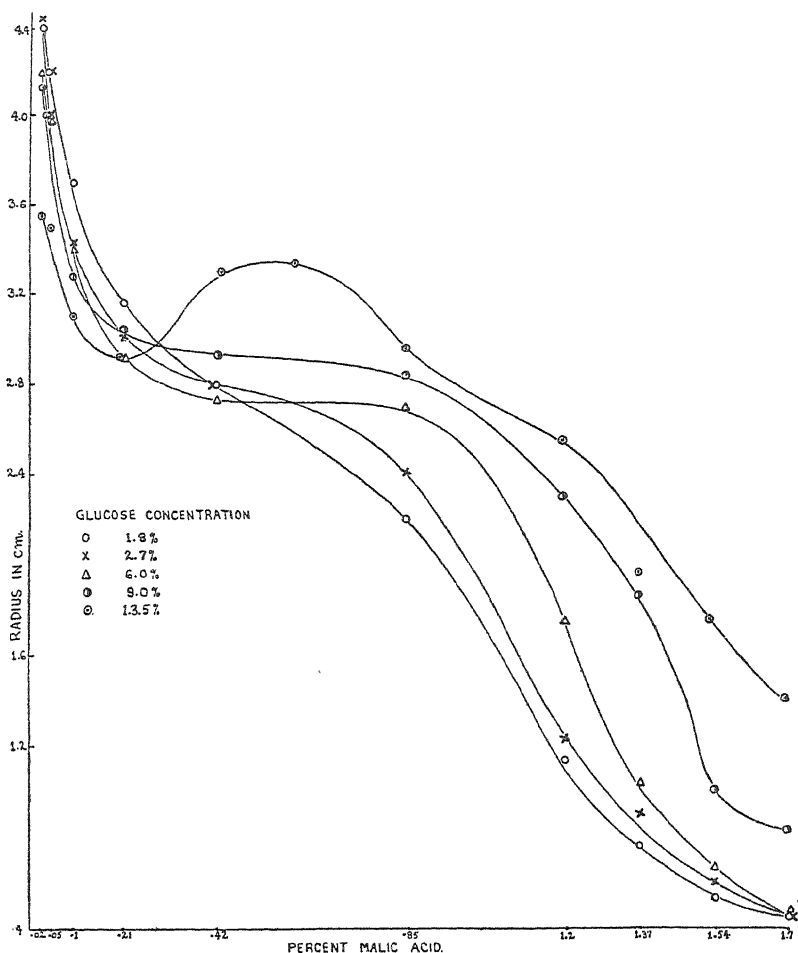


FIG. 7. Graph showing radial spread in relation to acid concentration for five different concentrations of glucose. *Phomopsis conglanensis*.

The effect of time on the course followed by individual curves is shown in Fig. 9 where radius of spread, observed on the fourth, sixth, and ninth days in the case of 0.025 A (unbroken line) and 0.05 A (broken line) is plotted against glucose concentration.

(c) *Diaporthe pernicioso*. Strain DHF.

In this case six concentrations of acid (0.025 A to 0.85 A) and seven concentrations of glucose (1.8 G to 17 G) were used, 42 combinations in all,

and the whole experiment was repeated twice. The data for total radial spread in 9 days are given in Table IV. The values given are in each case the mean values for three plates.

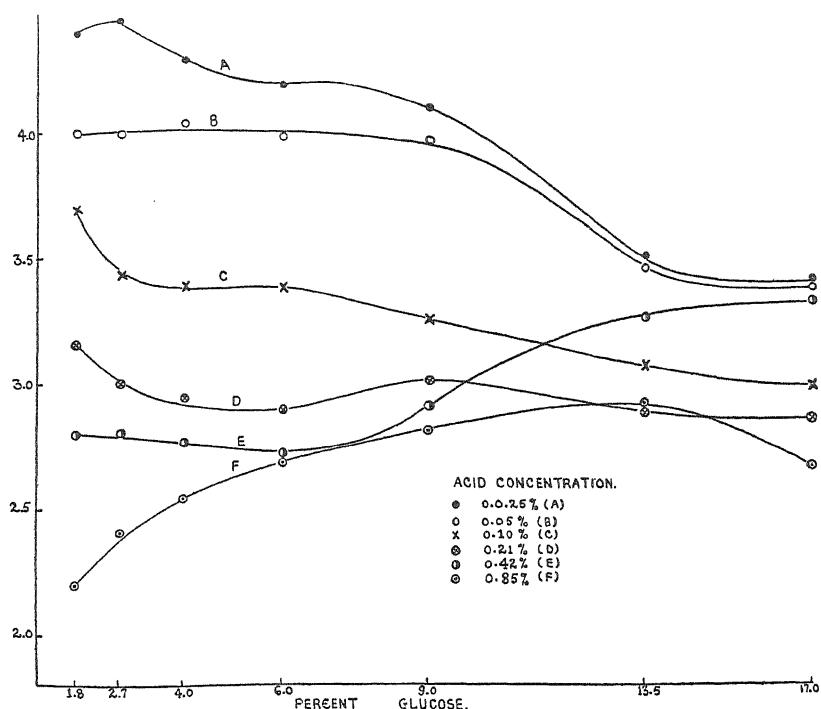


FIG. 8. Graph showing radial spread in relation to glucose concentration for six different concentrations of acid. *Phomopsis conglanensis*.

TABLE IV.

*Diaporthe pernicios*a, DHF. *Length of Radius in Relation to Varying Concentrations of Glucose and Malic Acid.*

Acid in grm. per 100 c.c.	Glucose in grm. per 100 c.c.						
	1.8.	2.7.	4.0.	6.0.	9.0.	13.5.	17.0.
0.025	33.0	32.0	32.8	35.0	38.0	39.5	40.5
0.05	31.1	32.0	31.3	33.8	37.5	39.0	37.3
0.10	31.0	32.0	31.0	33.1	35.5	39.0	39.5
0.21	27.8	27.5	28.0	30.1	32.1	32.5	33.0
0.42	17.3	20.6	21.6	23.5	22.2	23.5	19.1
0.85	13.0	13.6	13.7	13.8	16.9	16.5	16.8

The relation of radial spread to acid concentration is shown graphically in Fig. 10 where the curves A to F represent glucose concentrations ranging from 1.8 to 13.5 G respectively.

The following points are worthy of note :

1. The curves are of somewhat similar type, more or less convex to the base line.

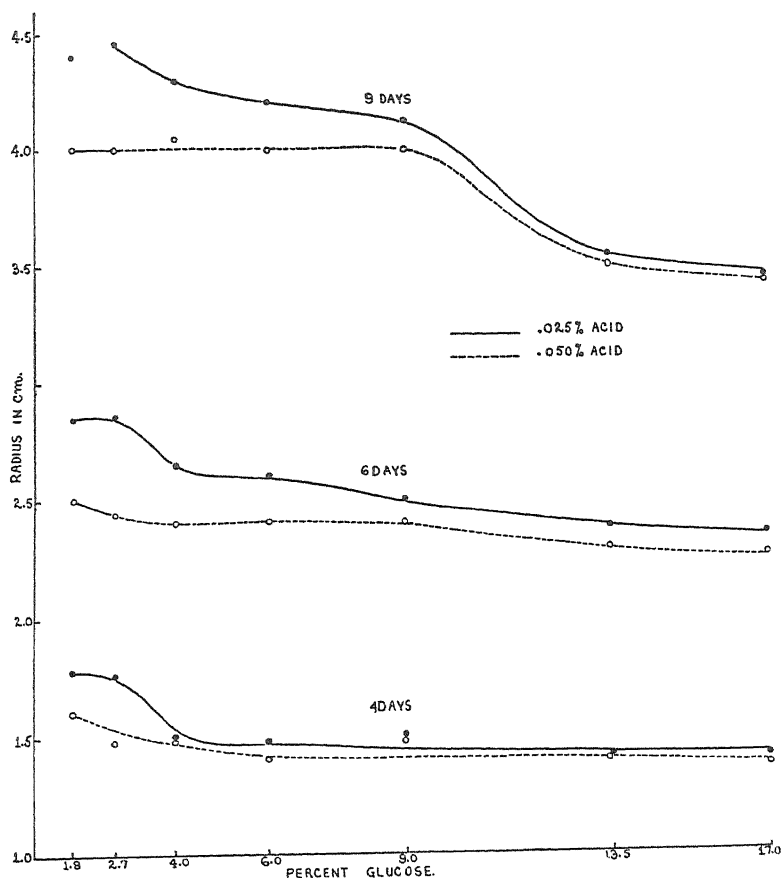


FIG. 9. Graph showing effect of age of culture on development of certain curves. *Phomopsis coneglanensis*.

2. For all concentrations of glucose the rate of spread falls continuously with increasing acid.

3. The curves A to F start from zero acid in the reverse order recorded for *P. coneglanensis* and *C. ludibunda*, CE, indicating that increasing glucose has in general the effect of increasing the rate of spread instead of lowering it. It will be noted that the curve F (13.5 G) intersects curves E and D indicating that at higher levels of acid high glucose tends to depress growth.

The curves obtained when data of radial spread are plotted against glucose concentration are given in Fig. 11. The curves for 0.025, 0.05, 0.1,

and 0.21 A (A-D) rise with increasing glucose instead of falling as in the case of *C. ludibunda*, CE and *P. coneglanensis*. On the other hand the

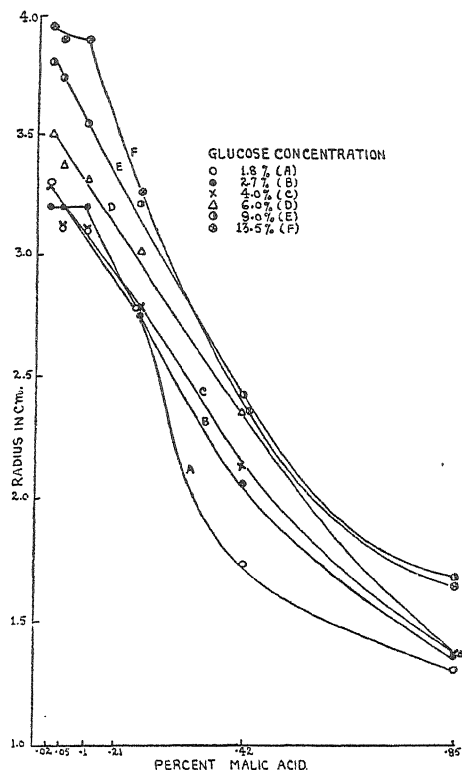


FIG. 10. Graph showing radial spread in relation to acid concentration for six different concentrations of glucose. *Diaporthe pernicioso*, DHF.

curves for 0.42 A and 0.85 A are of somewhat similar form in both cases, thus the curve E (0.42 A) rises to a maximum of about 9 G and then falls.

(d) *Cytosporina ludibunda*. Strain CA<sub>4</sub>.

In this case 70 combinations of acid and glucose were used (0.025–1.7 A and 1.8–17 G). Since very uniform results were obtained the experiment was not repeated. Data of total radial spread in 9 days are given in Table V, the values given being in each case mean values for three plates.

The results are presented in Figs. 12 and 13.

In Fig. 12 length of radius is plotted against concentration of acid for 7 concentrations of glucose. The points to be noted are as follows:

1. The curves are very regular and are convex to the base line

indicating that the rate of spread falls with increasing acid. It is seen from the shape of the curves that the rate of decline is steadily diminishing.

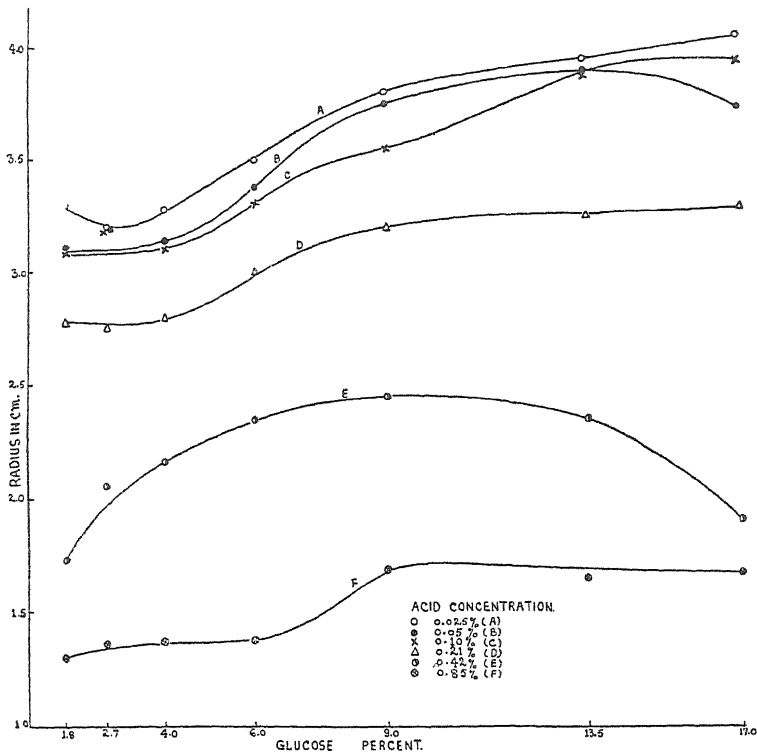


FIG. 11. Graph showing radial spread in relation to glucose concentration for six different concentrations of acid. *Diaporthe perniciosae*, DHF.

TABLE V.

*Cytosporina ludibunda*, CA<sub>4</sub>. Length of Radius in Relation to Varying Concentrations of Glucose and Malic Acid.

Acid in grm. per 100 c.c.	Glucose in grm. per 100 c.c.						
	1.8.	2.7.	4.0.	6.0.	9.0.	13.5	17.0.
0.025	29.8	29.6	29.1	25.5	18.0	12.8	11.5
0.05	22.0	20.3	20.5	18.1	15.1	11.8	11.0
0.10	19.8	18.5	18.0	16.1	14.0	12.0	11.0
0.21	16.3	15.5	14.5	13.1	11.5	9.5	8.6
0.42	10.5	10.0	9.5	9.0	8.6	7.0	5.5
0.85	5.1	5.0	5.5	5.5	5.1	4.6	4.0
1.20	3.5	3.5	4.0	5.0	4.0	3.8	3.8
1.37	3.5	3.5	3.5	3.5	3.5	3.3	3.1
1.54	2.8	2.8	3.0	3.0	3.0	3.5	2.5
1.70	2.5	2.5	2.5	2.5	2.5	2.5	2.0

2. The curves start from 0.025 A in the reverse order recorded for *D. pernicios*, DHF, but in the same order as that recorded for *P. conglanensis* and *C. ludibunda*, CE. The curves approach one another and

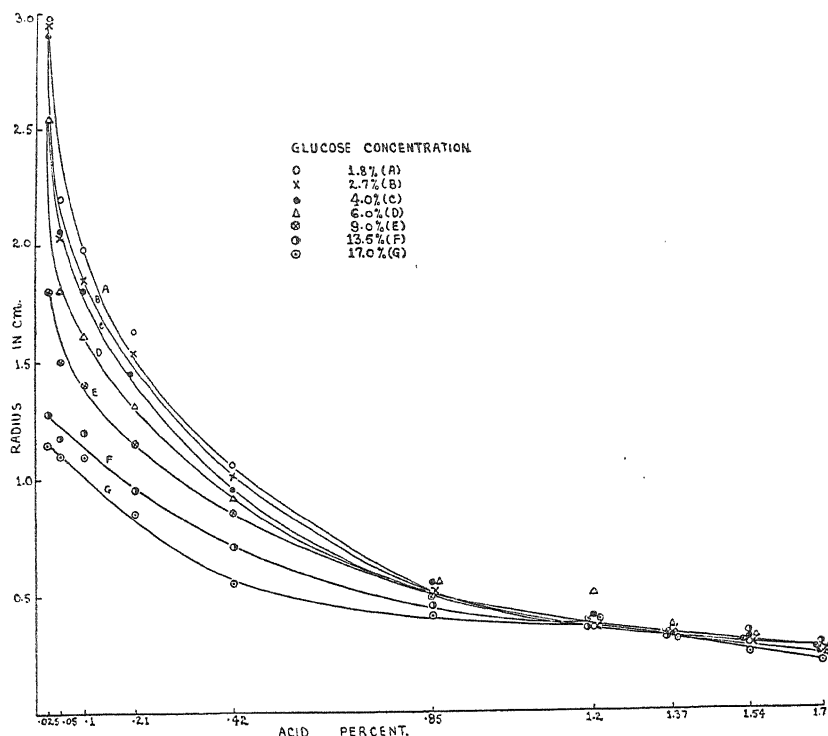


FIG. 12. Graph showing radial spread in relation to acid concentration for seven different concentrations of glucose. *Cytosporina ludibunda*, CA<sub>4</sub>.

tend to meet (1.2 A) but do not intersect, indicating that glucose has little effect on radial spread at relatively high concentrations of acid.

3. All the curves fall below that representing *D. pernicios*, DHF, 1.8 G (Fig. 10), indicating that the rate of spread is much lower at all combinations of acid and glucose.

In Fig. 13 length of radius is plotted against glucose for ten concentrations of acid. The curves thus obtained (A-J), unlike those recorded for *D. pernicios*, DFH, are descending curves, and are all very regular. The curve A for 0.02 A is of sigmoid form. The sigmoid tendency is still evident in B (0.05 A). The remaining curves tend to straighten out and to become horizontal. The three last curves (H, I, and J) are practically horizontal, illustrating further the point already mentioned that varied glucose has in this case little effect on radial spread when the level of acid is relatively high.

(e) *Cytosporina ludibunda*. Strain CC<sub>2</sub>.

In this case 42 combinations of acid and glucose were used (0.025–0.85 A and 1.8–17 G). Here, again, very uniform results were obtained.

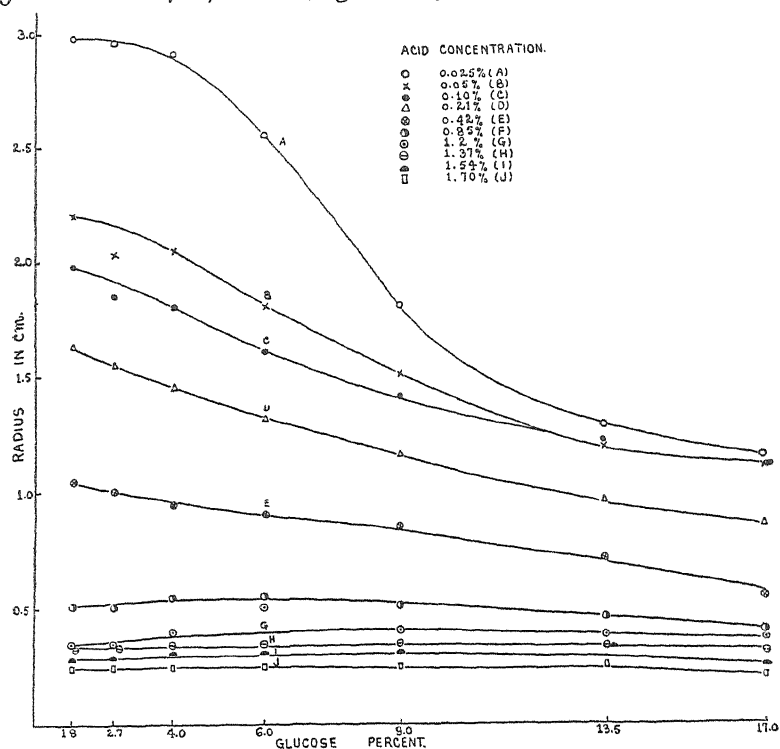


FIG. 13. Graph showing radial spread in relation to glucose concentration for ten different concentrations of acid. *Cytosporina ludibunda*, CA<sub>4</sub>.

Data of mean length of radius (3 plates) are given in Table VI. The data are plotted against acid concentration in Fig. 14 and against glucose concentration in Fig. 15.

TABLE VI.

*Cytosporina ludibunda*, CC<sub>2</sub>. Length of Radius in Relation to Varying Concentration of Glucose and Malic Acid.

Acid in gm. per 100 c.c.	Glucose in gm. per 100 c.c.						
	1.8.	2.7.	4.0.	6.0.	9.0.	13.5.	17.0.
0.025	6.0	8.5	9.1	6.6	3.1	2.5	2.0
0.05	10.1	13.5	11.5	6.6	4.0	2.5	1.8
0.10	12.5	16.1	15.3	6.8	3.8	2.5	1.6
0.21	11.8	14.0	12.1	5.0	3.0	2.1	1.3
0.42	9.0	8.5	5.5	3.0	2.0	1.0	0.5
0.85	2.0	1.6	1.0	0.5	No growth		



The curves presented in Figs. 14 and 15 differ from those given in Figs. 12 and 13 (*C. ludibunda*, CA<sub>4</sub>) in that curves for certain levels of glucose (Fig. 14 A-C) and acid (Fig. 15 A-D) show a rising limb, a

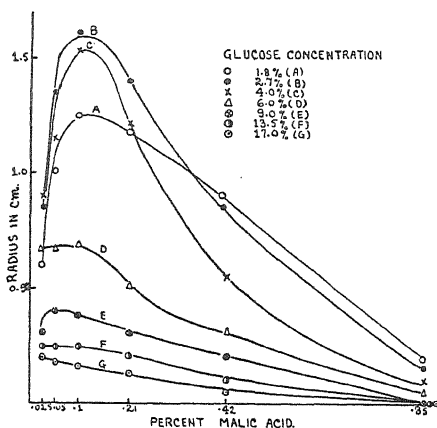


FIG. 14. Graph showing radial spread in relation to acid concentration for seven different concentrations of glucose. *Cytosporina ludibunda*, CC<sub>2</sub>.

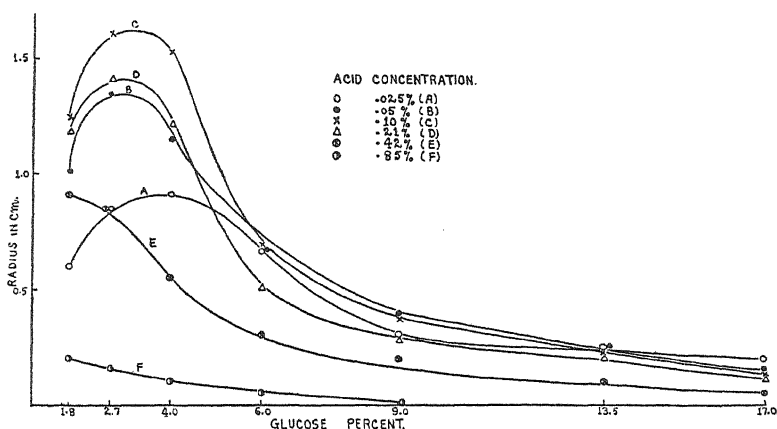


FIG. 15. Graph showing radial spread in relation to glucose concentration for six different concentrations of acid. *Cytosporina ludibunda*, CC<sub>2</sub>.

maximum point and a falling limb, indicating that at such levels radial spread increases with increasing acid or glucose, as the case may be, until a certain critical concentration is reached and then the rate of spread falls with increasing concentration until growth ceases.

(f) *Phomopsis vexans*.

The experimental results as in the case of *C. ludibunda*, CC<sub>2</sub> are based on 42 combinations of acid and glucose. The data are presented in

graphical form in Figs. 16 (glucose series) and 17 (acid series) where length of radius is plotted against concentration.

The curves for glucose shown in Fig. 16 should be compared with

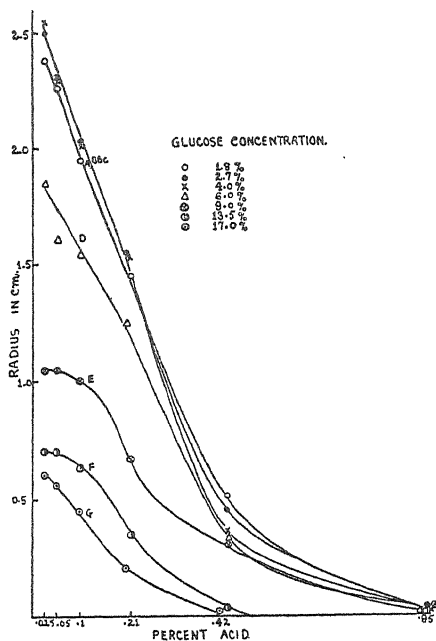


FIG. 16. Graph showing radial spread in relation to acid concentration for seven different concentrations of glucose. *Phomopsis vexans*.

those presented in Fig. 12 (*C. ludibunda*, CA<sub>4</sub>), which they closely resemble. The curves indicate that rate of spread falls with increasing acid at all levels of glucose. It is seen that growth is greatly retarded by high glucose. The curves for acid on the other hand, with one exception (0.42 A), at first rise to a maximum and then fall, resembling those given in Fig. 15 (*C. ludibunda*, CC<sub>2</sub>) in form, but not as regards the order in which they are found. No growth was observed in media with acid content exceeding 0.85 A.

(g) *Diaporthe arctii*.

This strain does not lend itself to detailed investigation because it is, as already mentioned, a strongly staling strain. Very little growth was observed after the seventh day in all the members of acid and glucose series. Observations made on the fourth day indicated that the rate of spread diminished with increasing acid.

(h) *Phomopsis citri*. Strains Jaffa 1, Jaffa 18, and Brazil 20.

The experimental work was limited to 12 combinations of acid and glucose (0.025, 0.05, 0.1, 0.21, 0.42, and 0.85 A), and (9 and 13.5 G). The results are expressed graphically in Fig. 18 where length of radius is

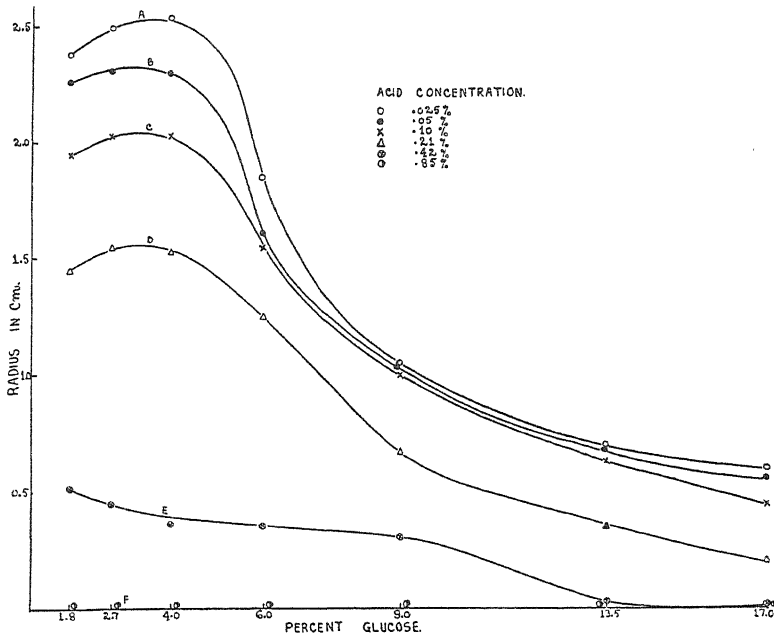


FIG. 17. Graph showing radial spread in relation to glucose concentration for six different concentrations of acid. *Phomopsis vexans*.

plotted against acid concentration for the three strains; curve A representing Jaffa 1 and Jaffa 18 for 13.5 G; curve B, Brazil 20 for 13.5 G; curve C, Jaffa 1 and Jaffa 18 for 9 G and curve D, Brazil 20 for 9 G. The curves A and C show a second maximum. The response of the strains (Jaffa 1 and Jaffa 18) to glucose and acid is therefore similar to that observed for *C. ludibunda*, CE, and *P. coneglanensis*. The position of the second maximum, however, varies with glucose concentration. Curves B and D (Brazil 20) do not show a second maximum, they resemble curves for *C. ludibunda*, CE, and *P. coneglanensis* obtained when lower concentrations of glucose are used (Figs. 3 and 7).

(i) *Diaporthe celsastrina*, *D. acerina*, *D. Beckhausii*, and *D. strumella*.

Since the strains were only recently received from Washington it has been impossible to investigate them in any detail. A preliminary test was made using 13.5 G and 7 concentrations of acid (0.025-0.85 A). The results are given in Fig. 19. Curves A, B, C, and D represent *D. celsastrina*,

*D. acerina*, *D. Beckhausii*, and *D. strumella* respectively. In each case the rate of spread falls with increasing acid. The intersection of the curves

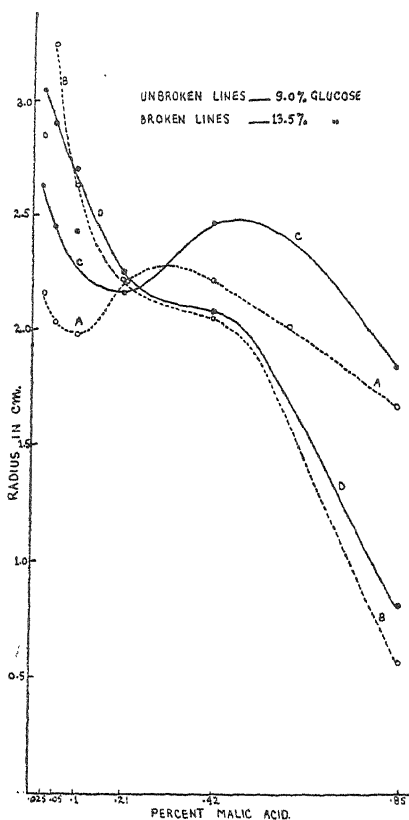


FIG. 18.

FIG. 18. Graph showing radial spread in relation to acid concentration for two concentrations of glucose. A, C. *Phomopsis citri*, Jaffa 1 and 18; B, D. *Phomopsis citri*, Brazil 20.

FIG. 19. Graph showing radial spread in relation to acid concentration using 13.5 G. A. *Diaporthe celastrina*; B. *Diaporthe acerina*; C. *Diaporthe Beckhausii*; D. *Diaporthe strumella*.

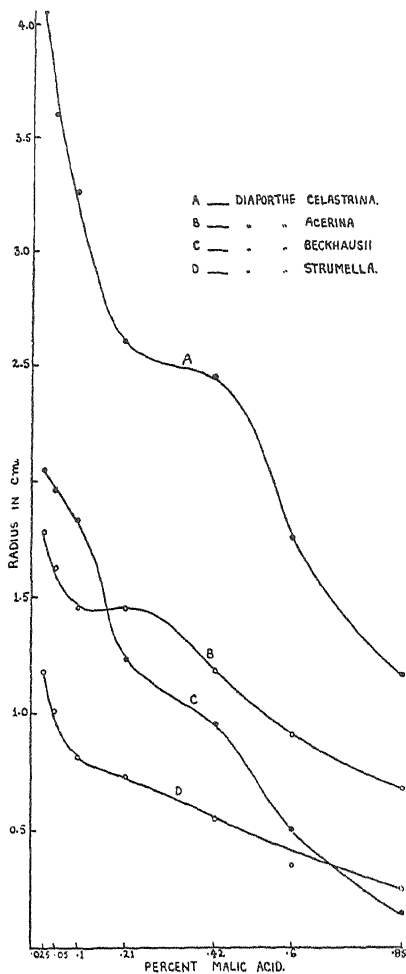


FIG. 19.

for *D. acerina* and *D. Beckhausii* is probably due to the fact that the former is a staling strain.

#### V. MALIC ACID AND SUCROSE.

In these experiments cane sugar was substituted for glucose in the standard medium. In order to compare the relative effects produced by

sugars other than glucose, an experiment was carried out in which first sucrose and then fructose was substituted for glucose in the standard medium, no acid being added. Equimolar solutions of sugars were used

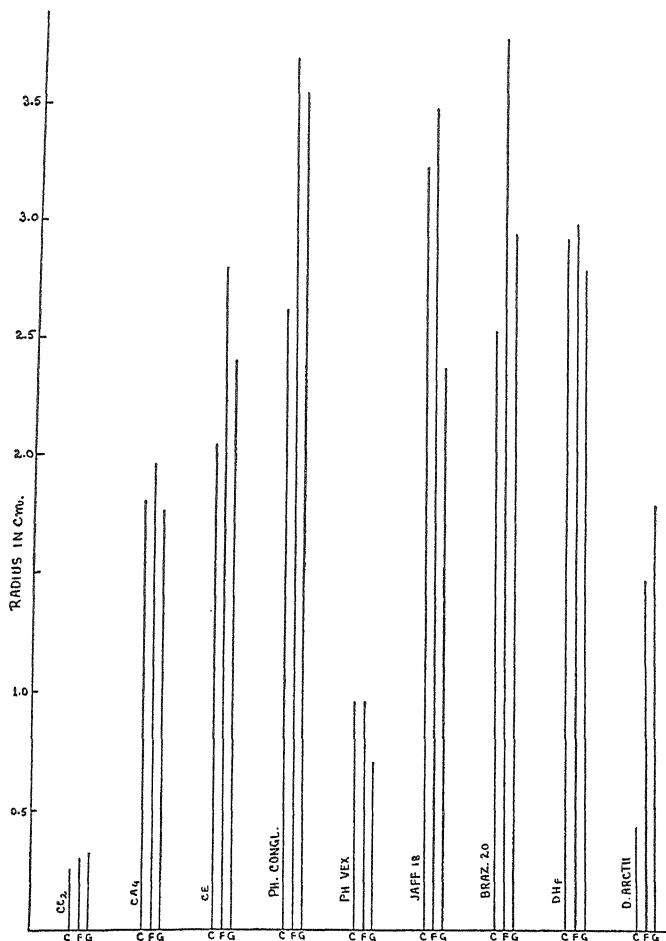


FIG. 20. Graph showing radial spread recorded for nine different fungi in relation to equimolar solutions of sugars. C. Sucrose; F. Fructose; G. Glucose.

corresponding to 13.5 per cent. glucose. Nine different fungi were tested, the results expressed in terms of length of radius observed on the ninth day are represented graphically in Fig. 20.

The vertical lines, given in groups of three in Fig. 20 for each strain tested, represent the values obtained for sucrose, fructose, and glucose respectively (C, F, and G). It is seen that sucrose has a depressing effect on growth in certain cases (*C. ludibunda*, CE, *P. coneglanensis*, *P. citri*, Brazil 20, and *D. arctii*); in others, the effect is not so marked (*C.*

*ludibunda*, CC<sub>2</sub> and CA<sub>4</sub> and *D. pernicios*a, DHF). In the case of *P. vexans* slightly increased growth was obtained with sucrose.

A single series was prepared using eight concentrations of acid and

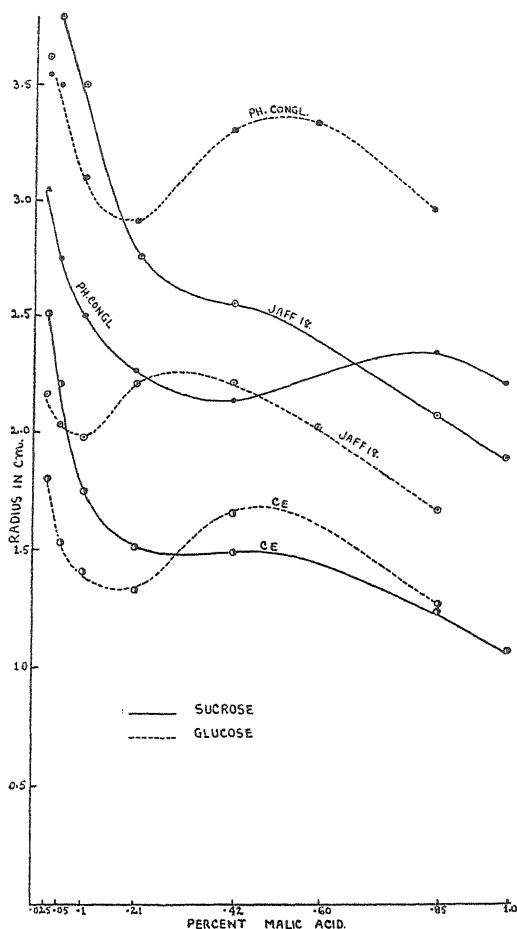


FIG. 21. Graph showing radial spread recorded for three fungi in relation to acid concentration for equimolar concentrations of sucrose and glucose (13.5 G).

sucrose equivalent to 13.5 G. Three fungi were tested, viz. *C. ludibunda*, CE, *P. coneglanensis*, and *P. citri*, Jaffa 18. The results are represented graphically in Fig. 21 by the unbroken lines. In the same figure curves for 13.5 G, selected from Figs. 3, 7, and 18, and represented by broken lines are given for the same strains.

It is seen from Fig. 21 that curves for sucrose and glucose do not follow a similar course. The strains show the following individual differences in their reaction to the two sugars: *P. coneglanensis*. Both curves show a second maximum (0.85 A, sucrose; 0.6 A, glucose). In media con-

taining sucrose radial spread is always less than it is in corresponding media containing glucose. *P. citri*, Jaffa 18. The sucrose curve did not develop a second maximum even when observations were continued beyond the ninth day. Radial spread was always greater in sucrose media. *C. ludibunda*, CE. At levels of acid not greatly exceeding 0.3 A there is more spread in cane sugar medium, but at this point the sucrose curve is intersected by the curve for glucose, owing to the fact that the latter rises to a second maximum, and then the reverse relation holds. Observations on the sucrose media were continued after the ninth day; on the twelfth day a second maximum was observed and showed considerable development on the fourteenth day. The effect of sucrose is therefore to delay the appearance of the second maximum.

## VI. MALIC ACID AND FRUCTOSE.

The results obtained when fructose is substituted for glucose in the standard medium are given in Fig. 21 to which reference has already been made. It is seen that the effect on radial spread varies from strain to strain: certain strains (*C. ludibunda*, CE, *P. vexans*, and *P. citri*, Jaffa 18 and Brazil 20) spread further in fructose medium; others (*C. ludibunda*, CC<sub>2</sub> and CA<sub>4</sub>, and *D. perniciososa*, DHF) spread nearly to the same extent; and others (*D. arctii*) do not spread as far. It is also seen from Fig. 21 that except in the case of *P. vexans* fructose favours spread more than sucrose.

In order to study the combined effect of fructose and acid on radial spread, seven concentrations of acid were used (0.025, 0.1, 0.21, 0.42, 0.6, and 0.85 A) and one of fructose (13.5 per cent.). Six different fungi were tested. The full data obtained for total radial spread in nine days are given in Table VII from which it is seen, in the case of sets where the level of acid was relatively high, that the replicates proved very irregular.

The relation between radial spread and acid concentration is shown graphically in Fig. 22.

The curves given in Fig. 22 with a single exception (*P. citri*, Jaffa 18) descend fairly sharply to the base line indicating that rate of spread is falling rapidly with increasing acid. In the case of *C. ludibunda*, CE, and CA<sub>4</sub>, *P. coneglanensis* and *P. citri*, Brazil 20, growth ceases at 0.6 A, and in the case of *D. perniciososa*, DHF, at 0.42 A. The curve for *P. citri*, Jaffa 18, differs from the remaining curves in that it shows a maximal point (0.1 A) and does not descend sharply to the base line. The results obtained with glucose and fructose respectively show quite clearly that fructose has a retarding effect on radial spread at all concentrations of acid in the case of *D. perniciososa*, DHF, *P. coneglanensis*, and *P. citri*, Brazil 20, but fructose favours spread at all concentrations of acid in the case of *P. citri*, Jaffa 18. In the case of *C. ludibunda*, CE and CA<sub>4</sub>

fructose favours spread at low levels of acid (up to 0.21 A) but at higher levels spread is very much retarded.

TABLE VII.

*Length of Radius in Relation to 13.5 per cent. Fructose and Varying Concentration of Acid.*

Strain.	Acid in grm. per 100 c.c.						
	0.025.	0.05.	0.1.	0.21.	0.42.	0.6.	0.85.
<i>Cytosporina ludibunda</i> , CE	31.0	28.0	19.5	12.5	4.0	No growth	No growth
	33.0	30.5	19.5	14.0	4.0	"	"
	33.0	30.5	22.5	13.5	No growth	"	"
<i>Cytosporina ludibunda</i> , CA <sub>4</sub>	15.5	13.0	10.0	8.0	3.5	"	"
	15.5	13.0	10.5	9.5	3.5	"	"
	15.5	13.0	12.0	No growth	No growth	"	"
<i>Phomopsis coneglanensis</i>	36.5	33.0	22.5	21.0	9.5	"	"
	37.0	35.0	23.0	21.0	No growth	"	"
	37.5	35.0	25.0	No growth	"	"	"
<i>Phomopsis citri</i> , Jaffa 18	31.5	37.0	38.0	35.5	27.0	21.0	18.0
	31.5	38.0	38.5	35.5	27.5	22.0	17.0
	33.0	37.0	—	35.0	28.0	22.0	15.5
<i>Phomopsis citri</i> , Brazil 20	23.0	21.5	21.5	19.5	14.5	No growth	No growth
	23.0	21.5	21.5	20.5	15.0	"	"
	23.0	22.0	—	20.0	15.5	"	"
<i>Diaporthe perniciososa</i> , DHF	26.0	22.0	13.0	9.0	No growth	"	"
	27.0	23.0	14.5	9.5	"	"	"
	28.0	24.0	15.0	10.0	"	"	"

Reference to Table VII shows that no growth was recorded for certain replicates. In order to find out if the inocula used were still alive and capable of growth, the inocula were transferred to standard medium in tubes. These tubes were examined periodically but no growth was observed.

It has been stated above that the majority of the fungi tested show no growth at 0.6 A, in order to ascertain whether or not growth is possible at this level of acid if the fructose content is lowered, three strains were tested on a series of media where 0.6 A was used in combination with twelve different combinations of fructose (1.8, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 F). The following results were obtained: In the case of *P. coneglanensis* and *C. ludibunda*, CE, a certain amount of growth was observed in media containing 9 F but no growth at levels higher than 10 F: in the case of *D. perniciososa*, DHF, growth occurred in media containing 10 F.

#### VII. MALIC ACID AND COMBINATIONS OF GLUCOSE, FRUCTOSE AND SUCROSE.

The study of the interaction between growth and media containing four variable chemical factors has been limited to a few experiments



whereby it was hoped to obtain some general information as to the effect of using combinations of sugars instead of only a single sugar.

(1) Glucose, fructose, and sucrose used in the ratio 1:3:2.5. The

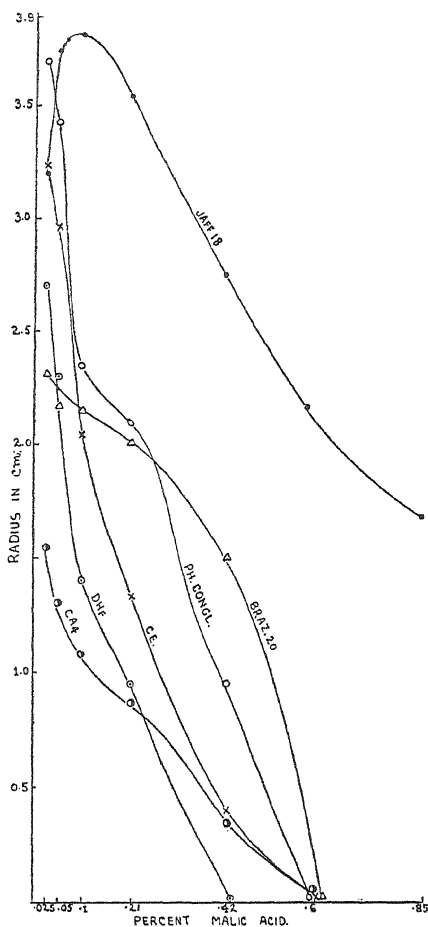


FIG. 22.

FIG. 22. Graph showing radial spread recorded for six fungi in relation to acid concentration using 13.5 F.

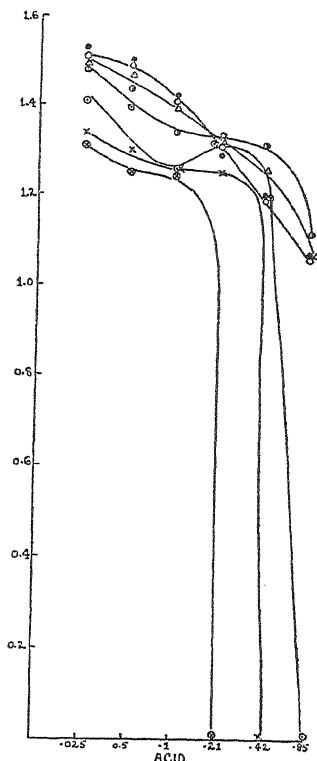


FIG. 23.

FIG. 23. Logarithmic curves representing the relation between radial spread and acid concentration for seven concentrations of combined sugars. *Cytophorina ludibunda*, CE.

actual proportions of these sugars used together with the glucose equivalent are given in Table VIII. In each case the glucose equivalent and the combination of three sugars are equimolar.

Only one fungus, *C. ludibunda*, CE, was used for the test. Data of total radial spread in nine days expressed in terms of logarithm of length of radius (mean of replicates) are given in Table IX. These values have

been plotted against acid concentration (logarithmic values) and the curves thus obtained, which may be compared with those given in Fig. 6 (glucose) are presented in Fig. 23.

TABLE VIII.  
*Concentrations of Combined Sugars.*

Glucose equivalent.	1.8 G.	2.7 G.	4.0 G.	6.0 G.	9.0 G.	13.5 G.	17.0 G.
Glucose	0.35	0.52	0.80	1.20	1.75	2.60	3.30
Fructose	1.04	1.56	2.30	3.50	5.25	7.90	9.80
Sucrose	0.80	1.18	1.80	2.60	3.97	5.95	7.40
Total	2.19	3.26	4.90	7.30	10.97	16.45	20.50

TABLE IX.

*Cytosporina ludibunda*, CE. *Logarithms of Length of Radius in Relation to Varying Concentrations of Acid and Combined Sugars.*

Malic acid per 100 c.c.	Total concentration of 3 sugars per 100 c.c.						
	2.19.	3.26.	4.90.	7.30.	10.97.	16.45.	20.50.
0.025	1.51	1.53	1.50	1.49	1.41	1.34	1.31
0.05	1.49	1.50	1.47	1.44	1.40	1.30	1.25
0.10	1.41	1.41	1.40	1.34	1.26	1.26	1.25
0.21	1.31	1.29	1.31	1.34	1.33	1.25	0.00
0.42	1.19	1.20	1.26	1.32	1.20	0.00	0.00
0.85	1.06	1.06	1.06	1.10	0.00	0.00	0.00

It will be seen from Fig. 23 that the curves resemble those obtained when glucose is used for the sugar ingredient (Fig. 6, CE). But whereas the glucose curves tend to run together at higher levels of acid and descend to the base line in the neighbourhood of 1.7 A the curves for combined sugars do not run together and reach the base line at different points: thus curve E reaches the base line at 0.85 A; curve F at 0.42 A and G at 0.21 A. Only one of the curves obtained as a result of plotting length of radius against acid concentration showed a second maximum, viz. the curve for 10.97 GFC (equivalent to 9 G) resembling curve E (9 G) given in Fig. 3.

(2) Glucose, fructose, and sucrose used in proportions found in the apple fruit: the proportions used are given in Table X.

TABLE X.  
*Proportions of Three Sugars Found in the Apple Fruit.*

	A.	B.	C.	D.	E.
Glucose	2.0	2.0	2.0	2.0	2.0
Fructose	6.5	7.0	7.0	6.0	5.0
Sucrose	4.0	3.0	2.0	1.0	1.0

The particular ratios (A-E) given, in Table X were chosen to represent proportions of sugars determined by chemical analysis for sets of apples differing with respect to age. Six concentrations of acid were used

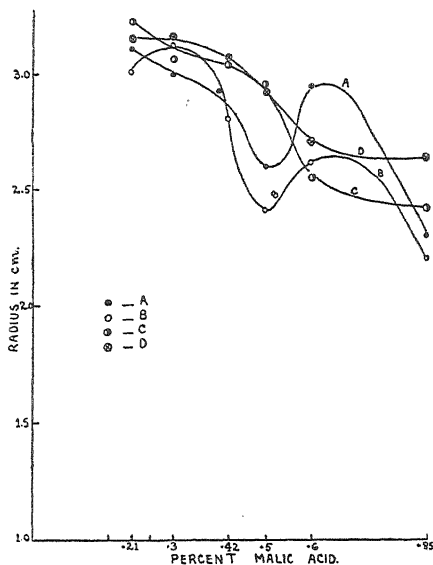


FIG. 24. Graph showing radial spread in relation to acid concentration for sugars combined in various ratios. *Phomopsis coneglanensis*.

(0.21, 0.3, 0.42, 0.5, 0.6, and 0.85 A) and only one strain, *P. coneglanensis*, was tested. The data obtained are presented graphically in Fig. 24, where length of radius is plotted against acid concentration. Curves for A, B, C, and D are given, the curve for E having been omitted because it closely resembles D.

It will be seen from Fig. 24 that the curves belong to the system of curves represented in Fig. 7 (*P. coneglanensis*). Only two of the curves (A, B) show a second maximum.

(3) Glucose, fructose, and sucrose found in Cox's Orange Pippin apples (2:6.5:4). This particular mixture of sugars was combined with seven different concentrations of acid (0.025, 0.05, 0.1, 0.21, 0.42, 0.6, and 0.85 A) and nine fungi were tested. The results obtained are presented in Fig. 25 where mean length of radius has been plotted against acid concentration as in the previous figure.

It is seen from Fig. 25 that the order of strains with respect to extent of radial spread varies with concentration of acid as shown by the fact that the curves for *P. coneglanensis* and *P. citri*, Jaffa 18, intersect at two points (0.54 A and 0.82 A), again the curve for *P. citri*, Brazil 20, intersects the curves for *P. coneglanensis*, *D. pernicios*a, DHF, and *C. ludibunda*,

CE, and so on. The curves for *P. coneglanensis* and *C. ludibunda*, CE, show a second maximum.

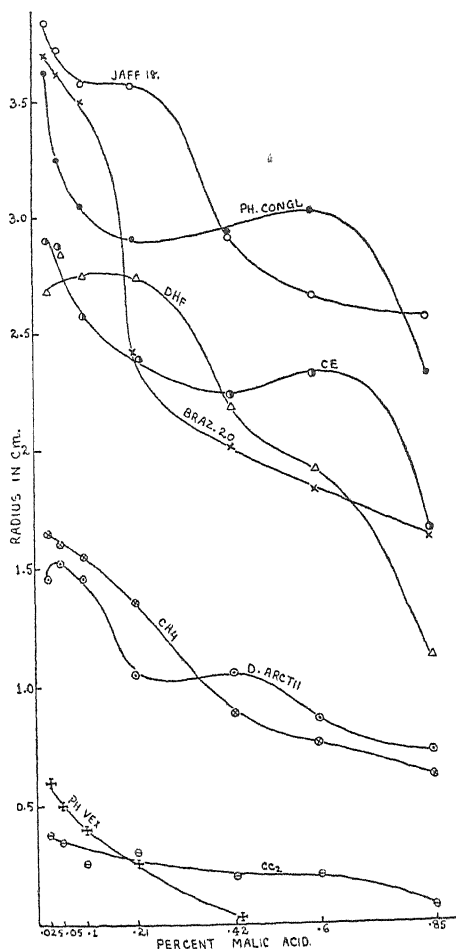


FIG. 25. Graph showing radial spread recorded for nine fungi in relation to acid concentration, using glucose, fructose, and sucrose in the ratio 2:6:5:4.

## VIII. DISCUSSION.

### *Salient Features of Results obtained.*

From the sets of curves presented in this paper it is seen that progress of fungal growth is greatly affected by varying concentrations of malic acid and sugar, and that nearly all the fungal strains investigated respond very differently to the same combination of the two variables. The response also varies with kind of sugar or relative proportions of different sugars (glucose, fructose, and sucrose) used in combination with acid. As a general rule it may be stated that both increasing concentration

of acid and increasing concentration of sugar tend to retard fungal progress. This point is illustrated in Fig. 12 (*C. ludibunda*, CA<sub>4</sub>) where the curves are of hyperbolic form, clearly indicating that radial spread is inversely proportional to concentration of acid. Such curves may be represented by formulae of the type  $r = K/X$  where  $r$  = radius of spread,  $X$  = concentration of acid, and  $K$  = a constant for any particular concentration of glucose. There are, however, several important exceptions to this rule. Thus, in the case of *D. perniciosus*, DHF (Fig. 11), the curves for acid tend to rise from near zero point with increasing glucose; and in the case of *C. ludibunda*, CC<sub>3</sub>, certain glucose curves (Fig. 14, A, B, C, and D) rise with increasing acid from near zero point. Such curves have a rising limb, a maximum point and a falling limb. Again, in the case of *C. ludibunda*, CE, and *P. cone-glanensis* the systems of curves presented (Figs. 3, 5, 7, and 8) are of an exceedingly complex nature. Thus in the case of the glucose curves presented in Figs. 3 and 7, starting from near zero point the curves descend with increasing acid and tend to follow a hyperbolic course until a certain critical concentration of acid is reached and then the curvature changes becoming parabolic. This change in curvature is not very marked when the level of glucose is very low but becomes more and more pronounced with increasing glucose. When the highest levels of glucose are reached the curves show a second maximum point. The curves, plotted on a logarithmic basis, given in Fig. 6 show clearly that they all belong to one family and the difference between the parts of the curves showing hyperbolic and parabolic curvature respectively in Figs. 3 and 7 is greatly emphasized. There is a tendency for each curve to be made up of two components which are nearly straight (*P. cone-glanensis*) or slightly curved (*C. ludibunda*, CE), and are inclined at an obtuse angle. The components may tend to meet at a point (*P. cone-glanensis*) or unite to form a loop (*C. ludibunda*, CE). The curves differ from one another in the angle made by the two components and the angle which these components make with the base line of the graph. The angles change in both cases with changing concentration of glucose. The nature of these curves suggests that the relationship of growth which applies when fungal growth in relation to low levels of acid is considered, tends to break down more and more as concentration of acid increases, and is replaced by a different relationship which could be represented by a different mathematical equation. An analogous system of curves is illustrated in the Technical Report of the Advisory Committee on Aeronautics (14). The curves in this case plotted to show the relation between inclination of chord and lift coefficient of aerofoils, show that at angles below 10° the curves are of the same general character, but at higher angles the curves differ widely. The change in the curves is attributed to a sudden alteration in the flow from an efficient to an inefficient type. Another instance where a relationship

is known to change has been described by Martin Geoffrey (12) who found that a relationship (Stokes Law) which governs the fluid speed required to support particles of matter becomes unstable when the particles have reached a certain size and then a linear relationship comes into operation, when the particles still further increase in size the second relationship in turn breaks down and is replaced by a parabolic one.

The phenomenon under discussion is not solely due to the interaction of acid and sugar because it is only encountered when certain fungi are used, such as *C. ludibunda*, CE, *P. coneglanensis*, and *P. citri*, Jaffa 18. It is therefore to be attributed to interaction between fungal strain and combination of acid and sugar. It is interesting to note that the saltants *C. ludibunda*, CA<sub>4</sub> and CC<sub>2</sub> do not show systems of curves belonging to the exceptional type, although they were derived from the same parent as *C. ludibunda*, CE. Curves showing a second maximum have been observed previously by certain pathologists working with simpler series. Thus Hopkins (11) when investigating the effect of acidity on the growth of the wheat scab organism found that growth increased with decreasing acidity, starting from pH 2.5, to a maximum at pH 4.0-4.5, and then decreased to a minimum at pH 5.0-5.5 and rose again to another maximum. Robins (13) found that when *R. nigricans* is grown on potato-dextrose agar it produced a double maximum curve, with a minimum between the two maxima at pH 5.2.

Sucrose in combination with acid may favour growth more than glucose, or retard growth according to the fungal strain employed. In the case of fungi (*C. ludibunda*, CE, and *P. coneglanensis*) showing a second maximum with glucose, the position of the second maximum is found at a higher level of acid and appearance of the second maximum is delayed. In the case of *P. citri*, Jaffa 18, the curve does not rise to a second maximum.

The results obtained with fructose are difficult to interpret. When fructose is present in media where acid is absent or in which the level of acid is relatively low, it in general favours fungal spread more than glucose; but when the level is relatively high then this relation is reversed. The peculiar growth reactions observed may not be due entirely to the fructose itself. Fructose is a somewhat unstable compound and may undergo change during sterilization or possibly some interaction takes place between fructose and acid when the ingredients of the medium are mixed together after sterilization. Nevertheless, whether the growth reactions mentioned above are due to fructose or decomposition products of fructose, they are not shown by all the fungi investigated, for example, *P. citri*, Jaffa 18, in which case no reversal of the growth reaction with increasing concentration of acid has been observed.

*Results Considered in Relation to Changes in Rate of Invasion of the Apple Fruit.*

It is evident from the brief survey of results given above that interactions of acid, sugar, and fungal strain are of a very complex character. Certain combinations of acid and sugar will tend to favour, others to depress, fungal spread, and the effect on spread varies with fungal strain. The work of Haynes and Archbold (5) has shown that acid and sugar content of apples varies widely with variety, and further that important changes in certain chemical constituents are associated with increasing age of fruit. The relative proportion of the three sugars (glucose, fructose, and sucrose) found in the fruit changes. The acid content is continually falling. Thus the acid, as estimated at the time of gathering, falls from 0.4 per cent to 0.0 per cent. in Worcester Pearmain; in Cox's Orange Pippin, from 0.6 per cent. to 0.2 per cent.; and in Bramley's Seedling, from 1.0 per cent. to 0.5 per cent. approximately. Total sugars vary within a narrower range. It will be seen that combinations of 9-13.5 per cent. sugar and 0.025-1.200 per cent. acid correspond very closely with levels of sugar and acid found in apples.

Horne (9) has recently attempted to co-ordinate the results obtained in this investigation and certain results derived from inoculation experiments. He states 'If fungal growth in the living apple is conditioned by such chemical factors as influence rate of spread in media, then the following general rule should apply to apples. With nitrogen and sugar-content varying within narrow limits, rate of invasion should increase and resistance fall with diminishing acid. Again, with nitrogen and acid varying within narrow limits, rate of invasion should tend to increase and resistance fall with diminishing sugar; rate of invasion and resistance should also vary with varying proportions of glucose, fructose, and sucrose. There are, however, important exceptions to this rule where fungi are concerned'. Horne suggests that strains showing hyperbolic curves will conform to the general rule. But in cases where a maximal point is found at some critical concentration of acid or sugar, not zero, resistance will first fall and then rise. Curves of this type have actually been recorded by Horne (8) for *F. fructigenum*, D and A, Cox's Orange Pippin. In the case of strains showing curves with a second maximum point, Horne (9) suggests 'In apples where the total sugars do not fall below nine per cent. of fresh weight, and the ratio  $F/(G + S)$  does not greatly exceed unity, resistance should fall with falling acid until a certain critical concentration is reached. With further fall in acid, resistance should first rise, and then fall rapidly as acidity approaches zero. Fluctuations in resistance may be expected with varieties of apple where acidity changes within a medium range of concentration'. Further experimental results obtained by Horne (8)

support this view. Curves for Bramley's Seedling apples, in which acidity falls from 1.0 per cent. to 0.5 per cent. approximately, show that resistance falls with increasing age of fruit. In Fig. 26 four curves are presented representing progress of invasion in the Cox's Orange Pippin (unbroken line) and Worcester Pearmain (broken line) varieties of apple by *P. coneglanensis* (curves marked A), and *C. ludibunda*, CE (curves marked B): the curves for *P. coneglanensis* have already been published (9). In the case of Cox's Orange Pippin where acidity changes from 0.6 per cent. to 0.2 per cent. approximately, curves A and B are of the fluctuating type. Curve A corresponds very closely with curves A and B given in Fig. 25 which represent spread in acid series containing sugars in proportions found in Cox's Orange Pippin. A second maximum is shown in curve A (Fig. 26) on October 8th. As regards curve B (Fig. 26) a second maximum is shown a week earlier. In the case of Worcester Pearmain where acidity changes from 0.4 per cent. to zero, approximately, curve B (*P. coneglanensis*) shows that resistance falls with increasing age of fruit (falling acid). Curve A (*C. ludibunda*, CE) shows a second maximum on October 1: it resembles that portion of the curve 13.5 G, Fig. 3, which is situated between 0.02 A and 0.3 A. In making comparisons of rate of fungal invasion of fruit and rate of spread in media, it must be borne in mind that, as far as apples are concerned, nitrogen content is liable to vary with variety, whereas the level of nitrogen has been kept constant throughout the cultural work. The effect of varying nitrogen on the form of curves representing radial spread still needs investigation.

#### *Results Considered in Relation to Attacking Power of Strains.*

Das Gupta (3, 4) working with several of the strains used in this investigation, has shown that the strains differ in their power of attacking apples and that the observed differences are in many cases significant. These strains, as the present author's results show, differ also in rate of spread in synthetic media of given chemical composition. The nine curves given in Fig. 25 which represent the relation between radial spread, as recorded for nine strains, and acid concentration, in the medium made up to resemble Cox's Orange Pippin apples, are all different. These strains may be grouped in order of decreasing activity in the media as follows: group 1, *P. citri*, Jaffa 18, and *P. coneglanensis*; group 2, *C. ludibunda*, CE; *D. perniciososa*, DHF, and *P. citri*, Brazil 20; group 3, *C. ludibunda*, CA<sub>4</sub>, and *D. arctii*; and group 4, *P. vexans* and *C. ludibunda*, CC<sub>2</sub>. This order is in agreement with Das Gupta's results: thus *P. coneglanensis* proved to be the most active strain tested; *C. ludibunda*, CE, less active; *C. ludibunda*, CC<sub>2</sub>, very weak, and so on.

When the strains within groups are considered individually the order



changes with increasing concentration of acid. The order of attacking power may therefore be expected to vary with variety of apple, if the varieties differ in acidity, and with age of apples since acidity falls with age of fruit. This view is borne out by the results obtained by Das Gupta

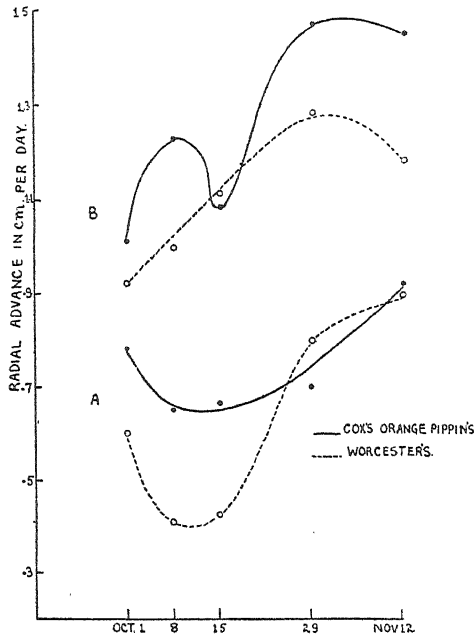


FIG. 26. Graph illustrating progress of fungal invasion in Cox's Orange Pippin and Worcester Pearmain varieties of apple. A. *Cytosporina ludibunda*, CE; B. *Phomopsis conglanensis*.

who compared attacking power of various strains as observed in different varieties of apple, apples of different age, and so on, using the method of analysis of variance. The analyses showed that both variety of apple (4) and age of fruit (3.4) may have a differential effect on attacking power of strains. For example, when Bramley's Seedling apples were inoculated early in the storage season (acid high), *C. ludibunda*, CC, proved more active than *C. ludibunda*, CA<sub>4</sub>, but when apples were inoculated on a much later occasion (acid low) the order of activity was reversed.

#### IX. SUMMARY.

The effect on the rate of radial spread of certain fungal strains of change in the concentration of acid and sugar in a standard nutrient medium has been investigated. It is found that the relation between spread and various combinations of these variables is affected by fungal strain, the kind of sugar, the concentration of the sugar, and if mixed sugars are used, the relative proportions of different sugars (glucose, sucrose, and fructose).

Systems of curves expressing this relation range from a system of hyperbolic curves to others which are exceedingly complex. The following specific examples may be given: (a) *C. ludibunda*, CA<sub>4</sub>. Spread is inversely proportional to acid concentration. (b) *C. ludibunda*, CC<sub>2</sub>. Spread increases with increasing acid or glucose until an optimal concentration of acid or glucose is reached and then decreases; (c) *C. ludibunda*, CE, and *P. coneglanensis*. At the lowest glucose concentration the curves are nearly exponential, spread decreasing with increasing acid. As the concentration of glucose is increased the curves become more and more complex, showing a second maximum at highest glucose concentrations. All the curves comprised in this system tend to intersect at a point which represents a concentration of acid where spread is independent of glucose concentration.

The various systems undergo modifications when sucrose or fructose or different proportions of glucose, fructose, and sucrose replace glucose in combination with acid. As a rule fructose favours growth at low concentrations of acid, but at higher concentrations of acid the reverse effect is found.

A series of media has been prepared where nitrogen, acid, and sugar are present in proportions found in apples at different times in storage life, as determined by chemical analysis. It is found that the curves representing radial spread of certain strains in relation to the varied chemical composition of the medium very closely resemble curves representing progress of invasion of the tissues of the fruit by the same strains.

The strains may be arranged in the following order of decreasing spread in the media used in this investigation. (1) *P. citri*, Jaffa 18; *P. coneglanensis*. (2) *C. ludibunda*, CE; *D. perniciosa*, DHF; *P. citri*, Brazil 20. (3) *C. ludibunda*, CA<sub>4</sub>; *D. arctii*. (4) *P. vexans*; *C. ludibunda*, CC<sub>2</sub>.

The order given above is also the order of power of attacking apples. When the strains in any one group are considered individually, the order varies with increasing acidity of media. A similar variation is also found with age of apple (acidity falling with advancing age) or with varieties of apple differing in acidity.

This work has been carried out under the supervision of Dr. A. S. Horne, to whom the author is greatly indebted for his invaluable help and criticism. The author's thanks are also due to Professor V. H. Blackman for the interest he has taken in this investigation, to Professor Sillick for advice on the mathematical side of the problem, and to Mr. H. Tooley for the photographs of graphical representations.

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# Studies in the Physiology of Wood-destroying Fungi.

## I. The Effect of Nitrogen Content upon the Rate of Decay of Timber.

BY

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### INTRODUCTION.

THE early history of wood preservation is full of references to the part played by albuminous substances in the decay of timber, and the preservative effect of metallic salts was ascribed to their action in coagulating the albumin. At a discussion on wood preservation held at the Institute of Civil Engineers in 1884, Professor Voelcher stated that 'the primary causes of decay of green wood were unquestionably the albuminoid substances, and all the older processes such as impregnation with corrosive sublimate . . . were based upon the principle that . . . the albuminoids were precipitated and rendered insoluble and incapable of acting as ferments'. Boulton, in reply, quoted a number of authorities and an experiment which he had himself carried out with a hard-boiled egg in order to refute this theory (1). The work of Hartig and others swept away this idea of coagulating the albumin to prevent decay and showed that the rotting of wood is due solely to the action of fungi, usually Basidiomycetes, which have no regard to the condition of the albumin in the wood.

Later the perishable nature of the sapwood of trees as compared to the durability of the heartwood was attributed to the presence of readily soluble food materials in the form of starch, sugars, and nitrogenous substances in the sapwood. But work by Lutz (7), Hawley, Fleck, and Richards (4), Sowder, and others referred to by Hubert (5), has shown that the principal cause of the durability of the heartwood of certain species is the presence there of soluble extractives and volatile oils which are toxic to the fungi. Lutz states that the specificity of fungi on certain hosts is due not so much to their requiring certain food materials which are present in those timbers, as to the existence in the heartwood of substances such as tannins, which are toxic except to fungi which are specialized and can resist them.

Thus, the possible influence of soluble food materials in wood upon its rate of decay by fungi, which feed largely upon the substance of the cell wall, has been somewhat overlooked. Gäumann (3) has found that the timber of spruce and fir felled at different times of the year in Switzerland shows varying resistance to fungal decay, but he ascribes this rather to different colloidal conditions of the cell wall than to varying quantities of soluble material in the cell.

The well-known practice of adding nitrogenous substances to accelerate decay of straw and other carbohydrate materials (a feature of the 'Adco' process) suggested that the activity of wood-destroying fungi might be affected in a similar way. As far as can be ascertained, no direct experiments have been carried out on this subject, though it was stated by Hartig that wood in stables, where it is exposed to contamination with urine, is prone to dry rot: he seemed to attribute this chiefly to the action of the alkali produced in the form of ammonia.

Horne (5) found that the rate of decay of apples by *Cytosporina ludi-bunda*, CE, under the circumstances of his experiments, was almost directly proportional to the nitrogen content of the fruit, and that the effect of adding nitrogenous manures to the trees was evident in the increased susceptibility to decay of the fruit.

#### *Object of the present investigation.*

The present investigation was carried out to determine whether the rate of decay of wood by fungi is influenced by the nitrogen content of the wood.

#### *Experimental procedure.*

It had been found in the laboratory testing of wood preservatives that the attack of fungi on wood is very much more certain when the wood is placed in contact with an actively growing, undisturbed culture of the fungus than when small pieces of a culture are cut out and placed on the wood.

Therefore, infection of the sample blocks was always carried out by placing them upon a culture of the fungus growing upon 2 per cent. malt extract agar in a modified form of the 'Kolle' culture flask which has already been described (2), and which contains in the neck a reservoir for moisture. The fungus is allowed to cover the surface of the medium before the blocks are introduced. In the first experiments the blocks were placed directly upon the medium, but it was found that they tended to become too wet for satisfactory growth of the fungi, and in later experiments they were supported on low glass frames so that the blocks came into contact only with fungus mycelium and not with the medium.

The timbers used were seasoned heartwood of Canadian-grown Sitka

spruce (*Picea sitchensis*) and outer wood of beech (*Fagus sylvatica*), the former timber being exposed to the attack of *Trametes serialis* Fr., and the latter to *Polystictus versicolor* (L.) Fr.

The blocks were sterilized by oven-drying at 100° C. for eighteen hours, and after being weighed were impregnated with the sterilized nitrogenous solution by means of a vacuum pump. They were allowed to soak in the solution for a few minutes and then removed and allowed to dry down in an oven at a temperature of about 50° C. till most of the free water had gone from the cells. Good absorption was always obtained in this way, the blocks taking up about their own weight of solution. The control samples were injected with sterile distilled water only, but otherwise received identical treatment.

The blocks were left exposed to fungus attack for two to three months at a temperature of 23° C. At the end of this period they were removed from the flasks, freed from adhering mycelium, oven-dried and re-weighed. The loss in weight which gives a measure of the amount of decay caused by the action of the fungus is obtained by subtracting the final weight from the initial weight; allowance being made for the dry weight of the added nitrogenous substance only where the concentration of the impregnating solution is greater than 1.0 per cent. The loss in weight is expressed as a percentage of the original dry weight of the wood.

In every case ten blocks were tested at each concentration and ten control blocks were used, and the results are expressed as the arithmetic mean of the percentages. The averaged results obtained in each experiment are given below, only in the last experiment are they given in full.

The moisture content of the blocks was estimated when they were removed from the flasks, and is based upon the oven-dry weight of wood.

### Experiment I.

Timber: Sitka spruce. Fungus: *Trametes serialis*.  
Duration of experiment: 8 weeks.

Conc. of sol. (%)	Loss in wt. %.	Moisture content %.
2.0 ammonium nitrate	9.66	158.4
1.0       "             "	11.34	157.2
0.5       "             "	12.95	159.8
0.25     "             "	14.82	148.8
0.10     "             "	19.36	102.3
Controls, distilled water	16.15	102.5

In the samples treated with concentrations of ammonium nitrate higher than 0.1 per cent., the moisture contents were considerably higher than in the controls and far above the optimum for growth of *T. serialis* in Sitka, which lies between forty and sixty per cent.

The pieces treated with 0.1 per cent. ammonium nitrate which had the same moisture content as the controls lost 3.21 per cent. more than the latter, an increase in the percentage loss of about 20 per cent., a figure which is probably just significant. The high moisture content of the blocks was due to their being in contact with medium from which the wood treated with the hygroscopic salt drew moisture.

### *Experiment 2.*

Timber: beech. Fungus: *Polystictus versicolor*.

Size of blocks:  $3 \times 1 \times \frac{1}{2}$  in.

Duration of experiment: 8 weeks.

Conc. of sol. (%).	Loss in wt. %.	Moisture content %.
5.0 ammonium nitrate	4.77	92.2
2.5       "       "	9.14	100.7
1.0       "       "	19.37	93.7
0.5       "       "	15.52	105.8
0.25      "       "	21.67	101.5
Controls, distilled water only	21.20	106.1

Again the moisture contents were considerably too high for satisfactory growth, but also the higher concentrations of ammonium nitrate appear definitely to retard growth of the fungus. There is a small but not significant increase in the rate of decay of the specimens treated with the 0.25 per cent. solution as compared with the controls.

### *Experiment 3.*

Timber: beech. Fungus: *Polystictus versicolor*.

Duration of experiment: 2 months.

Conc. of sol. (%).	Loss in wt. %.	Moisture content %.
0.2 ammonium nitrate	17.65	39.1
0.1       "       "	22.44	40.9
0.05      "       "	21.00	38.3
0.01      "       "	21.55	34.8
0.1 asparagin	20.56	38.5
0.1 peptone	19.13	35.4
Controls, distilled water only	19.12	39.2

In this case the blocks were supported on glass frames and the moisture contents remained suitable for active fungus growth. The percentage loss of weight was highest in the blocks treated with 0.1 per cent. ammonium nitrate, as in Experiment 1, being 22.4 per cent. as compared with 19.1 per cent. in the controls.

No further experiments were carried out on beech.



*Experiment 4.*Timber: Sitka spruce. Fungus: *Trametes serialis*.

Duration of experiment: 2 months.

Conc. of sol. (%)	Loss in wt. of blocks %	Final moisture content %
1.0 ammonium nitrate	12.05	46.3
0.5       "       "	15.08	48.6
0.1       "       "	12.90	40.8
0.05      "       "	11.91	38.9
Controls, distilled water	10.62	39.6
0.5 peptone	12.49	41.1
0.5 asparagin	14.28	44.5

In this experiment the blocks were supported on glass frames and the moisture contents were quite uniform. In every case the series which received additional nitrogen showed a higher percentage loss in weight than the controls which were injected with distilled water only. The highest losses in weight were shown in the series treated with 0.5 per cent. ammonium nitrate and with 0.5 per cent. asparagin, which lost 15.08 per cent. and 14.28 per cent. respectively, as compared with 10.62 per cent. in the controls.

*Experiment 5.*

The detailed figures of this experiment which yielded the most definite results are shown in full below:

Timber: Sitka spruce. Fungus: *Trametes serialis*.Size of specimens:  $3 \times 1 \times \frac{1}{2}$  in.

Duration of experiment: 12 weeks.

No. of block.	Init. % dry wt.	Impreg-nating sol.	Remarks.	Final oven-dry wt.	Loss.	Loss %	Final moisture content.
1	8.91	1.0 %	Little superficial	8.01	0.90	10.1	151.2
2	8.69	ammonium nitrate	fungus growth; sur-	7.70	0.99	11.4	136.1
3	8.60		face of blocks rotted	7.56	1.04	12.1	180.8
4	8.96			8.61	0.35	3.9	104.9
5	8.98			8.12	0.86	9.6	113.2
6	8.38			7.71	0.67	8.0	173.9
7	8.64			8.33	0.31	3.6	107.9
8	8.51			7.58	0.93	10.9	141.4
9	8.78			7.93	0.85	9.7	122.9
10	9.36			8.38	0.98	10.5	121.6
Av. loss % = 8.98: Av. m. c. = 135.4.							
11	8.58	0.5 %	Little superficial	7.31	1.27	14.8	119.0
12	9.02	ammonium nitrate	fungus growth; sur-	7.98	1.04	11.5	102.1
13	8.79		face of blocks rotted	8.17	0.62	7.1	132.5
14	8.68			8.26	0.42	4.8	117.5
15	9.35			8.41	0.94	10.1	141.8
16	9.04			7.82	1.22	13.5	146.4
17	8.74			7.47	1.27	14.5	144.6
18	8.82			7.69	1.13	12.8	129.1
19	9.37			8.09	1.28	13.7	119.6
20	8.98			8.08	0.90	10.0	94.8
Av. loss % = 11.8 %: Av. m. c. = 124.7.							

No. of block.	Init. % dry wt.	Impreg- nating sol.	Remarks.	Final oven-dry wt.	Loss.	Loss %.	Final moisture content.
21	8.44	0.25 % ammonium nitrate	Considerable superficial growth. Fruit bodies in Nos. 24 and 25.	7.42	1.02	12.1	136.1
22	8.46			7.20	1.26	14.9	155.4
23	8.78			7.52	1.26	14.3	101.9
24	9.12			7.81	1.31	14.4	93.8
25	8.45			6.88	1.57	18.6	95.0
26	8.71			7.18	1.53	17.6	111.3
27	9.00			7.40	1.60	17.8	106.9
28	9.10			7.41	1.69	18.6	69.8
29	8.42			6.68	1.74	20.7	101.9
30	8.98			7.72	1.26	14.0	93.9

Av. loss % = 16.30: Av. m. c. = 106.6.

31	9.22	0.1 % ammonium nitrate	Vigorous growth of fungus on surface of blocks. Fruit bodies on Nos. 31, 32, 35, and 40	7.02	2.20	23.9	55.8
32	8.56			5.88	2.68	31.3	85.9
33	8.82			5.94	2.88	32.6	57.9
34	8.60			5.78	2.82	32.8	60.6
35	8.42			6.40	2.02	24.0	55.2
36	9.08			7.62	2.06	22.7	66.0
37	8.38			5.47	2.91	34.7	63.1
38	8.49			6.68	1.81	21.3	68.5
39	8.86			5.91	2.95	33.3	62.6
40	8.92			6.78	2.14	24.0	85.8

Av. loss in wt. % = 28.06: Av. m. c. = 66.1.

41	9.17	Distilled water (controls)	Vigorous growth of fungus.	7.86	1.31	14.3	86.1
42	8.72			6.12	2.60	29.8	37.2
43	9.09			6.38	2.71	29.8	61.1
44	8.46			6.13	2.33	27.5	55.0
45	8.38			5.73	2.65	31.6	62.8
46	8.94			6.17	2.77	31.0	58.7
47	8.73			7.92	0.81	9.3	95.1
48	9.36			6.76	2.60	27.8	55.2
49	9.08			6.56	2.52	27.7	54.6
50	8.65			6.10	2.55	29.5	56.7

Av. loss in wt. % = 25.83: Av. m. c. = 62.3.

51	9.23	1.0 % peptone	Vigorous growth of fungus. Blocks completely rotted through.	5.12	4.11	44.5	81.4
52	8.81			5.29	3.52	40.0	63.0
53	9.03			4.82	4.21	46.6	72.2
54	8.99			5.46	3.53	39.3	66.3
55	8.62			5.58	3.04	35.3	62.6
56	8.80			5.33	3.47	39.4	68.9
57	8.52			5.02	3.50	41.0	66.7
58	9.09			5.06	4.03	44.4	64.0
59	9.11			5.50	3.61	39.6	66.2
60	9.05			5.60	3.45	38.1	69.9

Av. loss in wt. % = 40.82: Av. m. c. = 67.7.

61	9.09	1.0 % asparagin	Vigorous growth of fungus. Blocks completely rotted through.	5.62	3.47	38.2	65.1
62	9.22			6.02	3.20	34.7	64.3
63	8.97			6.09	2.88	32.1	49.6
64	9.13			5.13	4.00	43.8	71.0
65	8.62			5.79	2.83	32.8	64.3
66	8.57			5.54	3.03	35.4	61.0
67	9.30			5.28	4.02	43.2	60.4
68	8.68			5.46	3.22	37.1	66.9
69	8.53			5.32	3.21	37.6	65.0
70	9.24			6.02	3.22	34.9	60.8

Av. loss in wt. % = 36.98: Av. m. c. = 62.8.

In this experiment the moisture contents of the first three series of samples (those treated with 1.0, 0.50, and 0.25 per cent. ammonium nitrate) was much above that of the controls, and no comparison between the loss in weight figures is possible.

Considering the other series, in which the moisture contents were approximately equal, the samples treated with 0.1 per cent. ammonium nitrate lost 28.06 per cent. as compared with 25.83 per cent. in the controls, a difference that is possibly significant. The effect of adding an organic source of nitrogen was, however, very marked, the average loss in weight of the blocks treated with 1.0 per cent. peptone was 40.82 per cent. and of those treated with 1.0 per cent. asparagin was 36.98 per cent., a percentage increase of 57.6 and 43.2 respectively, as compared with the loss in the controls.

#### DISCUSSION.

In each of the three experiments with Sitka spruce detailed above, the effect of adding small quantities of nitrogen in the form of an inorganic salt was to increase the rate of decay, when moisture conditions in the treated and in the control blocks were comparable. The largest increase with ammonium nitrate was obtained in Experiment 4, where the controls lost 10.62 per cent. and the specimens treated with 0.5 per cent. of the salt lost 15.08 per cent., an increase of 42.1 per cent. over the percentage lost in the controls.

The figures obtained in the experiments with beech blocks are less significant; it is probable that the nitrogen content in the form of dried protoplasm, &c., is greater in the outer wood (sapwood) of beech than in the heartwood of spruce, and for this reason the later experiments were carried out on the latter timber.

The addition of an organic source of nitrogen in the form of peptone or asparagin markedly stimulated the growth of the fungus, and the loss in weight due to decay was increased from 25.8 per cent. in the controls to 40.8 per cent. in the peptone treated and to 37.0 per cent. in the asparagin treated, increases upon the control percentage of 57.6 per cent. and 43.2 per cent. respectively, which are undoubtedly significant. It has generally been found that the higher fungi respond more favourably to an organic than to an inorganic source of nitrogen, though they are capable, to a certain degree, of utilizing ammonium and nitrate compounds for their nitrogen requirements.

When the composition of fungus mycelium in relation to the composition of the wood, from which it is deriving its food material, is considered, it is not surprising that the addition of nitrogenous compounds has a favourable effect upon growth. The chemical composition of fungus

mycelium is still a matter of discussion, and doubtless it varies considerably in different species, as well as in different parts of the same fungus plant. The cell walls of woody forms such as *Fomes* give the phloroglucin reaction for lignin, and some recent work by Thom and Phillips (9) indicates that a large percentage of lignin may be present in species such as *Trametes pini* (54.08 per cent.) and *F. igniarius* 36.9.5 per cent. But most workers have agreed that a considerable percentage of the cell wall consists of a chitin-like body, which is stated to be identical with animal chitin. Now chitin contains just over 6 per cent. of nitrogen, and in addition to this must be added the nitrogen in the cell contents. Few exact figures are available for the nitrogen content of fungus mycelium, particularly as regards wood-destroying fungi. Thorpe quotes figures for the protein content of various fleshy fungi which expressed as percentages of the dry weight are as follows:

<i>Psalliota campestris</i>	47.5 per cent.
<i>Polyporus ovinus</i>	11.9 „
<i>Coprinus comatus</i>	35.1 „

Calculating the nitrogen as 1/6 of the protein the figures are roughly 8.0 per cent., 2.0 per cent., and 6.0 per cent. respectively, indicating a wide variation. Turning now to the nitrogen content of wood, Schorger (8) states that this varies from 0.1 to 0.3, seldom exceeding the latter figure. The Sitka spruce used in the present investigation was found to contain 0.04 per cent. of nitrogen, while the nitrogen content of the mycelium of *Polystictus versicolor*, which had been removed from the surface of a block of decayed beech wood, was 0.68 per cent. of the dry weight at 100°, and that of the mycelium of *T. serialis* scraped from the surface of an agar culture was 1.7 per cent.<sup>1</sup> Comparing the concentration of nitrogen in *T. serialis* with that in the wood upon which it was feeding, it will be seen that it was roughly forty times as great, indicating a considerable accumulation of nitrogen by the fungus. It is therefore obvious that wood substance considered as a food material for fungi is very deficient in nitrogen. Therefore the addition of nitrogen in a form readily available to the fungus, such as peptone, might be expected to stimulate growth, and such has been found to be the case.

In practice it has often been observed that timber decays more rapidly in rich soils than in poor, and this may in part be accounted for by the greater supply of nitrogen resulting in a greater fungus population of such soils, which leads to more intense infection and to the infiltration of nitrogenous substances into the timber, rendering it more readily decomposable.

<sup>1</sup> The author is indebted to Dr. Janet Brown of the Imperial College of Science for kindly carrying out the nitrogen determinations by microchemical methods.

## SUMMARY.

The early theories regarding the influence of albuminous substances upon the decay of wood are briefly reviewed.

Experiments are described in which small blocks of the wood of Sitka spruce (*Picea sitchensis*) and of beech (*Fagus sylvatica*) were treated with various concentrations of nitrogenous substances and exposed to the attack of the fungi, *T. serialis* and *P. versicolor*, growing under controlled conditions.

The results obtained showed that ammonium nitrate in low concentrations slightly increases the rate of decay of the spruce wood by *T. serialis* and possibly of beech by *P. versicolor*. Treatment of Sitka spruce with organic sources of nitrogen caused a large increase in the loss of weight due to decay. Specimens treated with 1.0 per cent. peptone losing 40.82 per cent., as compared with a loss by the controls over the same period of 25.83 per cent.

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# Further Studies on Transport in the Cotton Plant.

## II. An Ontogenetic Study of Concentrations and Vertical Gradients.<sup>1</sup>

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With nine Figures in the Text.

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### I. INTRODUCTION.

EARLIER papers from this station have described work leading to the conclusion that in the cotton plant transport, from the foliage region, of carbohydrates, of nitrogen, and also of phosphorus and potassium takes place via the phloem, and that this movement of material has a gradient basis. There seem to be two ways in which a gradient of mobile material between a region of supply and a region of utilization might determine the observed rapid movement of materials along the

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intervening phloem track, namely (1) by the diffusive spread of each mobile substance independently, the rate of spread for all mobile substances being accelerated by some mechanism in the phloem; (2) by mass flow of sap, containing all the mobile materials, from the region of higher turgor pressure (region of supply) to the region of lower turgor pressure (region of utilization). Although the latter view has recently been very ably reformulated and defended by Münch (9), while the former is at the basis of the well-known streaming hypothesis of de Vries (2) and, later, of Curtis (1), neither can at present be regarded as more than working hypotheses to guide experimental attack on the problem.

As suggested in the previous paper of this series (8), a simple test of the two alternatives would be to endeavour to obtain evidence of the movement of different materials in opposite directions along the same region of phloem. The normal upward movement of soil nutrients towards the foliage region of the cotton plant does not provide such a test, for this upward movement appears to take place via the wood. Further experiments along these lines have, however, been carried out and will be presented in a subsequent paper in which the two alternative possibilities for phloem transport will be more fully discussed. In the present paper we are concerned with the question whether the observed gradients of mobile material in the phloem of cotton are consistent with our working hypothesis that movement is determined independently for each by its concentration gradient.

A simple relation was found (7) between the observed concentration gradient of sugars in the bark, and the rate and direction of carbohydrate transport. For nitrogen, however, it was found (6) that, with the possible exception of the residual nitrogen fraction, the concentration gradients of the soluble organic nitrogen fractions were in the opposite direction to the normal downward transport. In order to maintain the original hypothesis it was necessary to postulate (6) a storage component, masking the dynamic component of translocatory material, in the observed gradients. This postulate was based on the facts that (1) arresting longitudinal movement abolished the originally positive vertical gradient of sugars, but steepened the negative gradient of crystalloid nitrogen; (2) that reversal of the normal direction of movement along the main axis was accompanied by a reversal of the gradient of sugar concentration in the inner region of the bark, and a steepening of the originally negative gradient of crystalloid nitrogen. We interpreted these changes in the observed crystalloid N-gradient as being due to the abolition or reversal of a positive dynamic gradient, superimposed on a relatively permanent storage gradient of greater magnitude. It seemed that while the concentration of the storage component might vary with age and history of the plant, yet regions at different vertical levels might normally differ in a characteristic way, the



concentrations being greater in the lower regions which are, of course, the older.

Although the further question of characterizing the storage and dynamic components in chemical terms or in terms of localization in different tissues of the bark had to be left undecided, some evidence was obtained suggesting that the storage component might consist mainly of asparagine. Thus the results of radial subdivision of the bark indicated that whereas asparagine predominated in the ray tissues, the other organic crystalloid fractions, amino acid and residual N, were present mainly in the sieve-tube region. Again, the negative gradient of crystalloid N in the bark as a whole was mainly a negative gradient of asparagine. Owing to the fact that the amount of sap available did not always permit the full fractionation of the crystalloid N, the contribution of the amino acid and residual N fractions to the storage and dynamic components, respectively, could not be decided. For phosphorus and potassium, which we found to be, like nitrogen, transported downwards from the foliage region via the phloem, similar dynamic and static components had to be postulated (8), since the total vertical gradients were mainly negative.

The work described in the present paper represents an approach from a slightly different angle. In discussing with Dr. F. F. Blackman the general conception of a vertical gradient in a storage component masking a translocatory gradient, he suggested that this would be given a broader basis if it were found to be supported by an ontogenetic study of the vertical distribution of concentrations in the main axis. In order to make the information bearing on transport as complete as possible it was decided to include phosphorus, potassium, and calcium as well as carbohydrates and nitrogen fractions in the determinations made. At the same time the use of a newer method of estimating inorganic nitrogen, requiring smaller volumes of sap, made it possible to determine all the crystalloid N fractions on every sample. Information as to the vertical gradients of residual N is therefore more complete than in previous work.

The chief points on which information was sought were (1) how do the vertical gradients in the bark behave as development proceeds? (2) what evidence does the time series give of storage of any particular class of compound?

## II. PLAN OF WORK.

The plants were sown on November 14, 1928. Flowering and fruiting were prevented by removal of flower buds. Vegetative branches also were removed as they appeared, so that the plants consisted of the main axis bearing main axis leaves and leafy fruiting branches.

The population was graded at the beginning of January and representative samples, of twenty-five plants each, drawn for collection at

(roughly) monthly intervals. Two such samples were taken at each collection. The main axis was subdivided into zones, as shown in the table below:

TABLE I.

*Scheme for Subdivision of Main Axis.*

Collection No.	1	2	3	4
Days from sowing	70	98	131	159
Root zone R.	Main root from ground level to 9" below ground level.			
Stem zone 1.	Ground level to 1st fruiting branch, 7 internodes.			
" " 2.	1st to 8th fruiting branch, 7 internodes.			
" " 3.	8th to 15th fruiting branch, 7 internodes.			
" " 4.	15th to 21st fruiting branch, 6 internodes.			
" " 5.	21st to 28th fruiting branch, 7 internodes.			

The four or five immature internodes of stem zones 3, 4, and 5 at collections 1, 2, and 3, were not sampled. These were still elongating and the bark could not well be separated from the wood.

Collection was made between 8 a.m. and 9 a.m. The stems were cut into the correct zones and the bark separated from the wood. The roots were dug up, washed clean, laterals removed, and the main root trimmed to 9 in. from ground level. They were then dried with filter paper, and bark and wood were separated. After determining the fresh weight of the samples, subsamples were taken in the usual way for moisture determination, while the bulk of each sample was at once frozen at  $-15^{\circ}\text{C}$ .

On the sap subsequently expressed from the frozen material the following were determined: reducing sugars, sucrose, total crystalloid N, asparagine N, amino acid N, ammonia N, nitrate N, total solids, and weight of water per 5 c.c. sap. The methods used were those described previously (6, 7) with the exception that ammonia N and nitrate N were determined by the aspiration method at room temperature of Sessions and Shive (10). For some of the collections determinations were made also of the freezing-point depression and the electrical conductivity.

The following determinations, using the methods previously described (6, 7, 8), were made on the dried material: acid hydrolysable polysaccharides, total N (including nitrates), total phosphorus, total potassium, total calcium.

## III. DRIFT OF CONCENTRATIONS AND GRADIENTS.

(a) *General Considerations.*

Presentation of concentration gradients offers no special difficulty. These are in all cases given as grm. or mg. per 100 grm. water. The case is different when we wish to discuss accumulation or storage. With one exception (the root zone for the first collection) the data obtained enable

us to calculate the total amount of each substance present in each zone at each collection. Increase or decrease in the content for any zone is thus known without the uncertainty that arises when we know only the composition per 100 grm. fresh weight or dry weight or ash weight. Should we find a decrease following an increase it would seem reasonable to speak of remobilization for utilization elsewhere and to consider part of the previous increase as representing temporary storage. But where we have increases only we cannot decide without some further information how far these represent (1) normal increase due to tissue growth, or (2) accumulation and storage. It is commonly assumed that either fresh weight or dry weight provides a valid basis of reference for this purpose, so that increase or decrease relative to dry weight or fresh weight may be taken as evidence of accumulation or depletion. Clearly this would be true only when no substance other than the one under examination showed either accumulation or depletion, or when the individual accumulations of some exactly balanced the depletions of others. Thus no generally valid basis of reference can be constructed from the weights either of all or of some of the materials present in any region of the plant. We can at most obtain evidence of the increase of any one substance relative to certain others which may also be increasing or decreasing. The significance of any observed increase so measured will vary with the particular reference line chosen.

In this paper we make use of three reference lines as follows: (1) fresh weight, chosen because of its common use and as a rough measure of bulk growth; (2) weight of water, chosen as being the basis for the sap concentration gradients; (3) weight of total carbohydrate material, chosen as representing, firstly, material derived by photosynthetic activity from the air as against materials derived from the soil, secondly, material which, in the form of cell walls, &c., normally accumulates in stem tissues as they age.

A survey of the situation from this point of view is given in Fig. 1, which shows the drift in time of the values for these reference lines and also for total nitrogen, phosphorus, potassium, and calcium. Since we are interested in relative changes the values plotted are the common logarithms of the actual weights per zone, and the vertical scales have been displaced so as to make the points for all materials at collection 1 coincide. The figure is for the bark and wood of the first two stem zones, since these cover the whole period of the experiment. As in previous papers, we take, as our estimate of total carbohydrate material, dry weight—5.7 N—ash.

All materials increase rapidly during the first period, and much more slowly during the second period, while in the third period there is either no change or only a slight rise or fall. Of the three reference lines, water content increases least, and total carbohydrate most, while fresh weight is intermediate. The increasing divergence is due, no doubt, to accumulation

of polysaccharides and thickening of cell walls as the tissues mature, and naturally is much more marked in the wood. Considering now the behaviour of total N with respect to the three reference lines, a very significant difference between bark and wood is manifest. In the bark total N increases more rapidly even than total carbohydrate, so that if we regard the carbohydrate increase as an accumulation we find a still more marked accumulation of nitrogen. In the wood, on the other hand, total N increases less rapidly than even fresh weight, so that although there is an increase in concentration per 100 grm. water, it seems doubtful whether we may speak of accumulation.

For phosphorus the increase in the bark is, up to collection 3, intermediate between that of total N and total carbohydrate; in the last period there is a decrease. Thus there is evidence for storage of phosphorus in the bark. In the wood the increase of phosphorus is not far below that of total carbohydrate, and again there is a decrease in the last period. Thus, relative to nitrogen, storage of phosphorus is much more marked in wood than in bark.

Potassium shows no sign of accumulation, the increase being, in every case, very close to that for water. The increase of calcium in the bark is about the same as that of fresh weight, but continues right up to the last collection. In the wood there is marked accumulation of calcium up to collection 3, after which there is little change.

These differences in the behaviour of the four elements will be discussed more fully later in connexion with the vertical gradients.

### (b) *Carbohydrates.*

#### (1) *Gradients.*

The sugar gradients in bark and wood are shown in Fig. 2. The total sugar gradients in the bark are consistently positive from the highest region of stem sampled down to the roots. The gradients are steep at collections 1 and 2 but show a marked fall at collection 3. This change in gradient forms a very close and suggestive parallel to the marked fall in rate of growth of the basal stem zones shown in Fig. 1, and would seem to be a good confirmation of the general position that rate of carbohydrate transport is determined by magnitude of sugar gradient in the phloem. As in all previous observations on cotton, the total sugar gradient is almost entirely a gradient of sucrose, and the change in this gradient during development is again due to change in sucrose. Reducing sugars appear, however, to contribute to the gradient from lowest stem zone to root zone. A further point of interest is that while the general level of sucrose concentration falls as the plant matures, that of reducing sugars rises.

In the wood there appears to be no consistent vertical gradient of

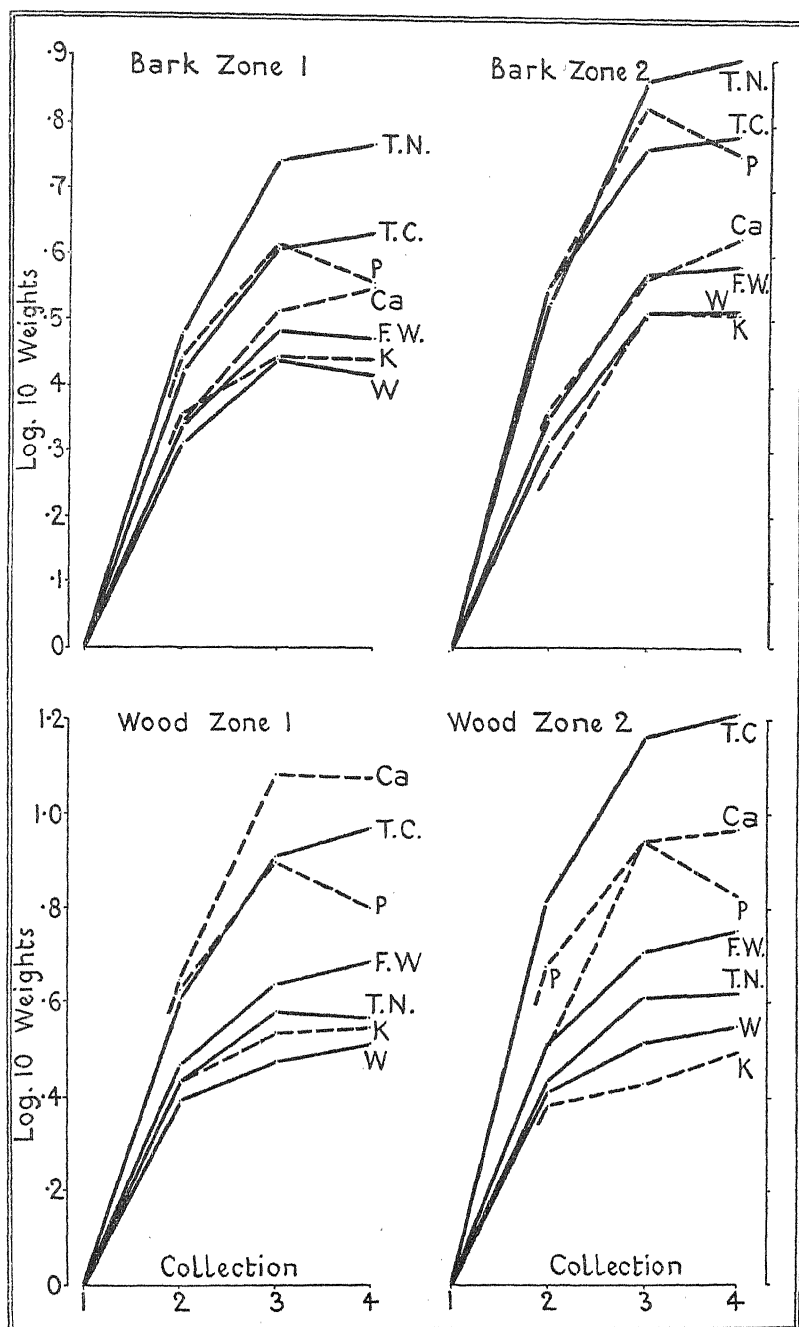


FIG. 1. Relative increments of different materials in bark and wood. The values plotted are the increments, since collection 1, in the common logarithms of the weights, per zone, of each material. W. = water; F.W. = fresh weight; T.C. = total carbohydrate material; T.N. = total nitrogen; P. = phosphorus; K. = potassium; Ca. = calcium.

total sugars; the sucrose gradients are, on the whole, negative, and the reducing sugar gradients positive. There is a marked rise in general level of concentration up to collection 2 but little change thereafter. In as far

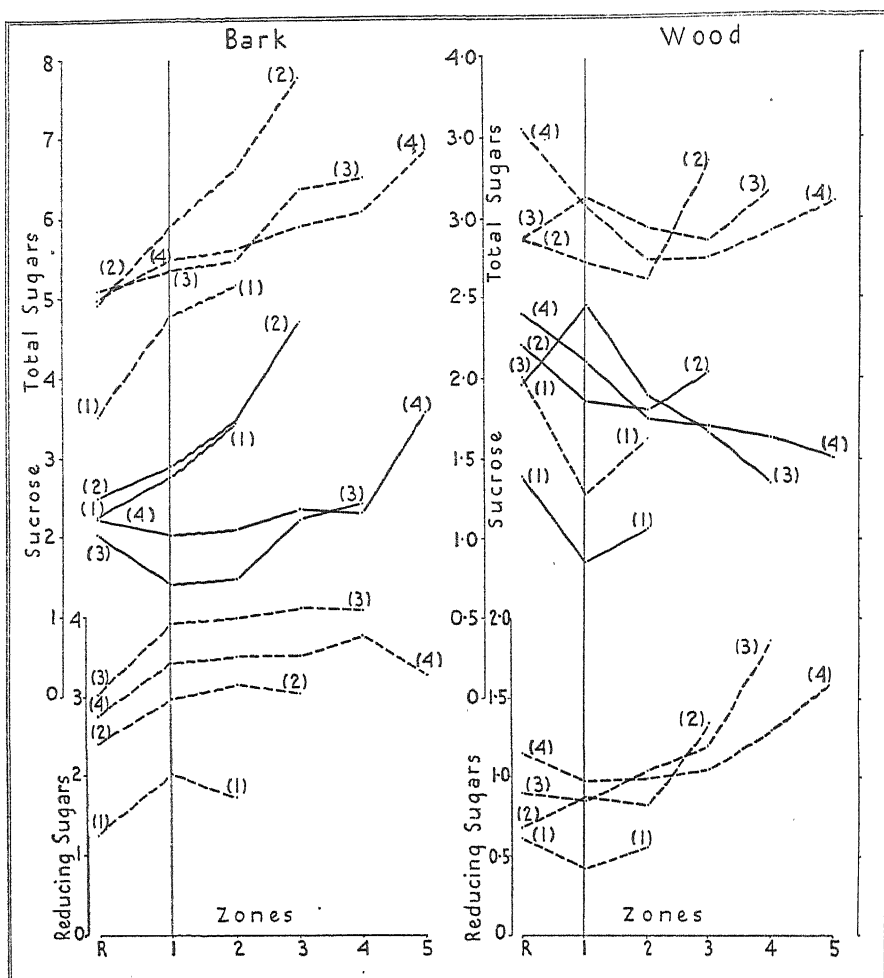


FIG. 2. Sugar concentrations in bark and wood, gm. per 100 gm. water. Curves for successive collections are numbered (1), (2), (3), (4).

as these changes in bark and wood are an *index* of changes in the sieve-tubes and in the living cells of the wood respectively, the gradient of sugars, and in particular the gradient of sucrose into the wood, should fall off markedly in all zones as the plant ages and also as we approach the base of the stem. This is in agreement with the fact that the rate of increase of total carbohydrate material in each wood zone falls rapidly with age and falls steadily also as we approach the base.

TABLE II.

	Rate of increase in total carbohydrate material.			Mean gradient of total sugars from bark to wood.			Mean gradient of sucrose from bark to wood.		
	% per week.								
	Period. 1-2.	Period. 2-3.	Period. 3-4.	Period. 1-2.	Period. 2-3.	Period. 3-4.	Period. 1-2.	Period. 2-3.	Period. 3-4.
Zone 1.	35	14.5	3.5	3.35	2.62	2.22	1.49	0.02	-0.54
Zone 2.	47	17	3	3.78	3.27	2.71	2.02	0.63	0.03
Zone 3.		23	6		3.94	3.33		1.63	0.63
Zone 4.			16			3.26			0.89

The agreement is, of course, only in general drift, and cannot be stressed, but it is suggestive.

## (2) Carbohydrate reserves.

The acid-hydrolysable polysaccharides, which may be regarded as the main storage component of the total carbohydrate gradients, increase in concentration from above downwards, i.e. they show a negative gradient. In Table III we give the polysaccharide values per 100 gm. total carbohydrate material, and it will be seen that even on the basis of this, the highest of the three possible reference lines (cf. Fig. 1), there is a steady accumulation during development, especially in the bark.

TABLE III.

*Acid-hydrolysable Polysaccharides gm. per 100 gm.  
Total Carbohydrate Material.*

Collection No.	Bark.				Wood.			
	1.	2.	3.	4.	1.	2.	3.	4.
Root zone R.	17.3	23.6	37.5	37.9	15.9	20.5	28.3	28.9
Sem zone 1.	18.2	20.3	25.1	30.2	15.4	19.3	27.2	27.2
" " 2.	17.8	19.1	26.4	28.2	14.0	17.5	23.6	21.5
" " 3.		17.4	25.1	24.9		19.3	23.4	20.0
" " 4.			22.0	23.7			24.1	18.2

## (c) Nitrogen.

### (1) Gradients.

The vertical gradients of total N, protein N, and crystalloid N are shown in Fig. 3. In the bark, where, as we have seen, total N accumulates with age, the general level of concentration rises very markedly with time, and the stem gradients are negative. There is, however, a positive gradient of total N from stem to root. In the wood, on the other hand, the general level of concentration changes little and the gradients are, on the whole, positive, though small. Accumulation of crystalloid N in the bark is even more marked than that of protein N and it is mainly this fraction which

determines the negative gradient. In the three basal stem zones and the root zone this accumulation of crystalloid N appears, at collections 3 and 4,

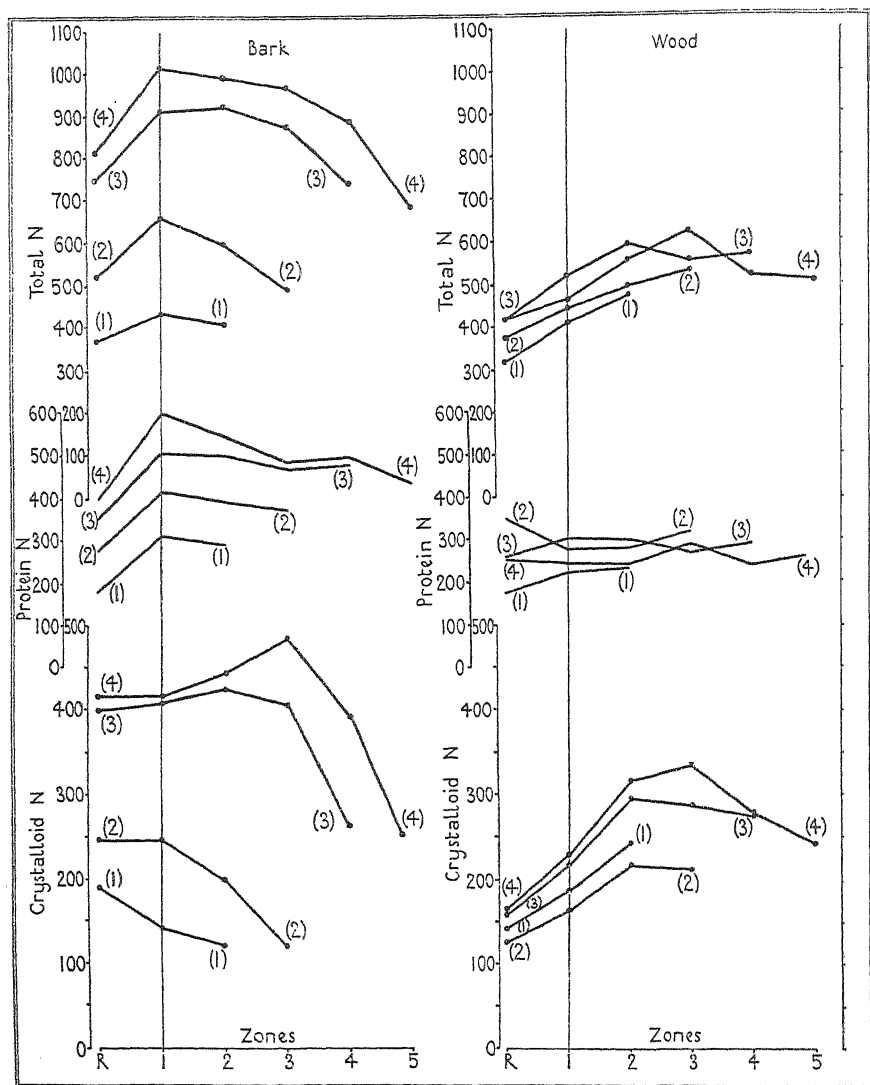


FIG. 3. Concentrations of total, protein, and crystalloid nitrogen in bark and wood. Mg. per 100 gm. water.

to have reached a saturation value, so that the negative gradient is flattened out in these regions. The results strongly support the view that the crystalloid N includes an important storage component which up to a point accumulates with age and is in consequence normally present in higher concentration in the more mature zones.



The curves for the individual crystalloid N fractions (Fig. 4) indicate that in all probability this storage component is asparagine N. This

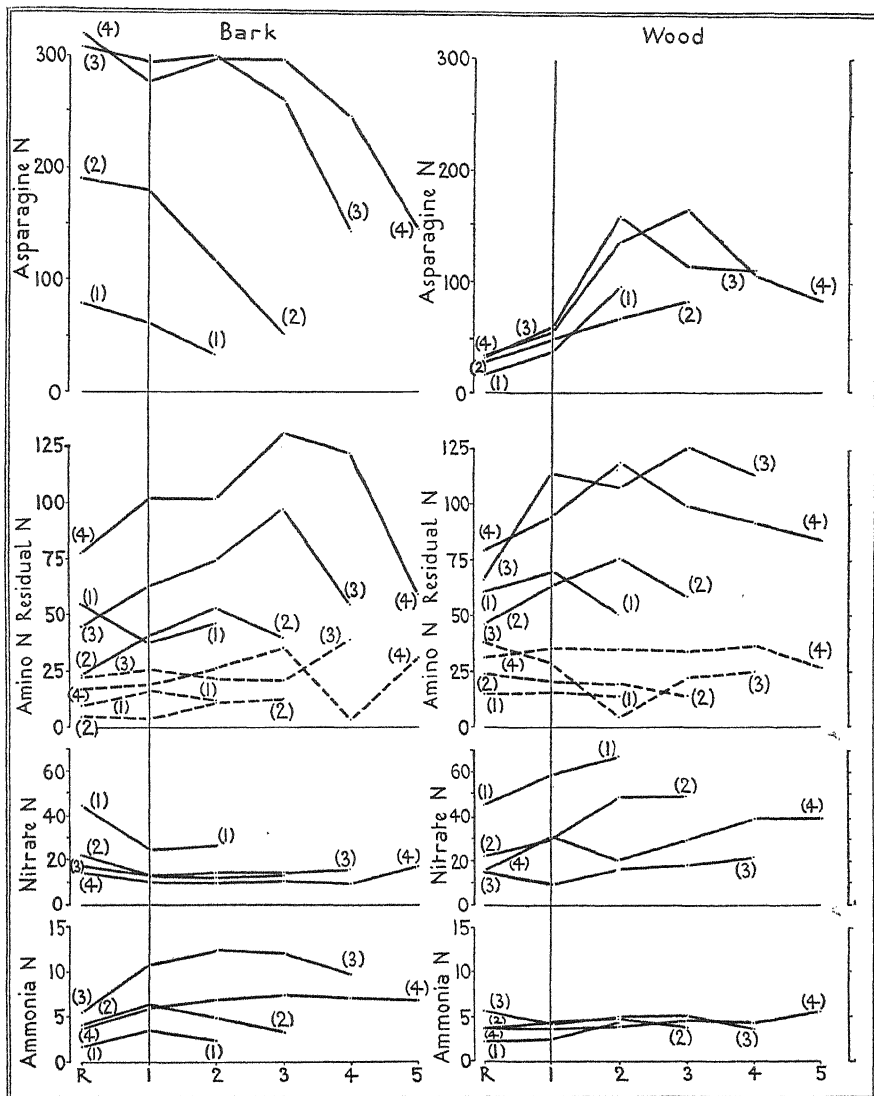


FIG. 4. Concentrations of crystalloid nitrogen fractions in bark and wood. Mg. per 100 gm. water.

fraction accounts for the greater part of the negative gradient at the earlier collections, while its tendency to reach a level value in the lower zones at the later collections allows of the appearance of a slight positive net gradient in these zones at the last collection. Amino acid N increases

with age, but the general concentration level is low and there is little sign of any vertical gradient. Nitrates fall with age and show no gradient. Ammonia N first rises and then falls with age, but again shows only small vertical gradients. Residual N, on the other hand, shows, with one exception (Stem zone 1 to Root zone at collection 1), a consistent positive gradient from near the apex downwards. If, as seems possible, this fraction, or some considerable part of it, represents the dynamic component of the crystalloid N gradient in the bark, the fall in concentration from the next highest to the highest zone sampled would suggest that nitrogen is moving from about the centre of the foliage region via the phloem both downwards towards the root and upwards towards the less mature apical regions. If so, we should have movement of carbohydrate in some upper zones in opposition to the movement of nitrogen, for the sugar gradient is positive up to the highest zone sampled.

One further point of interest may be noted. The residual N gradient shows no sign of that diminution with maturity that was so characteristic of the sugar gradients. If nitrogen movement is determined mainly by the gradient of residual N, then downward movement of nitrogen from the foliage region must be proceeding as rapidly in the later as in the earlier stages of development, while downward movement of carbohydrates is slowing off. A study of rates of movement during the life-history might throw light on this question, which has an important bearing on the general problem of the independence of the movement of different materials along the phloem.

The gradients in the wood do not call for much comment. Asparagine has, except in the upper zones, a positive gradient and, especially in the basal zones, there is only a small increase during development. Residual N in the wood on the whole resembles residual N in the bark and maintains a concentration level about as great. The concentration in the sieve-tubes (cf. 6) should be very much greater than in the bark as a whole, but the concentration for the living cells of the wood will also be greater than that for the wood as a whole, so that, while the general parallelism suggests transport from bark to wood as residual N, no close estimate of the gradient can be made.

## (2) *Storage.*

We concluded earlier (p. 124) that as the plant grows total nitrogen accumulates in the bark, and from a consideration of the changes in concentration (per 100 grm. water) of the nitrogen fractions we have suggested that this is due to storage partly as protein, but partly also as a crystalloid fraction, namely asparagine. Fig. 5 enables us to test the three important nitrogen fractions protein N, asparagine N, and residual N in the same way as we tested total N earlier (Fig. 1). The values plotted are for

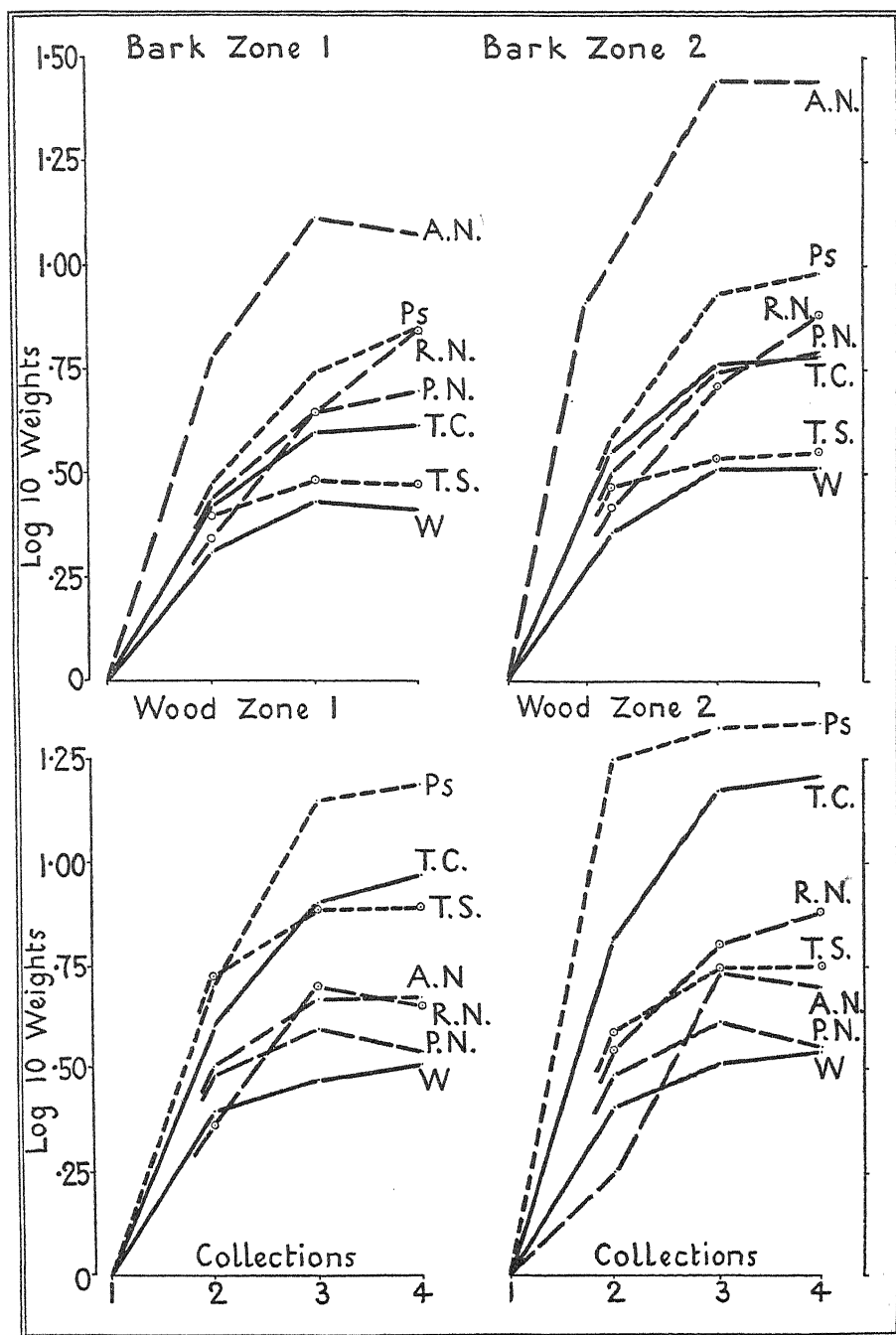


FIG. 5. Relative increments of carbohydrate and of nitrogen fractions in bark and wood. The values plotted are the increments, since collection 1, in the common logarithms of the weights per zone, of each material. W. = water; T.C. = total carbohydrate material; T.S. = total sugars; Ps. = polysaccharides; P.N. = protein N; A.N. = asparagine N; R.N. = residual N.

the first two stem zones and represent the increases in the common logarithms of the actual weights per zone from collection 1 onwards. Two reference lines—water content and total carbohydrate material—are given and, for comparison, polysaccharides and total sugars also.

In the bark the increase of protein is as great or greater than the increase of total carbohydrate (the highest of the reference lines), but this is far exceeded by the increase of asparagine. Even polysaccharides lag far behind asparagine. Residual N at first increases only a little more rapidly than water content; later it reaches, and finally exceeds, the increase of protein, but still remains well below the asparagine value. The results confirm our general picture of the storage of asparagine in the bark tissues as these age, leading to a negative vertical gradient of total crystalloid N in the main axis. Assuming that residual N represents mainly translocatory nitrogen, the increased concentration during development must mean continued export of nitrogen from the leaves coupled, perhaps, with a diminished rate of utilization for new tissue growth in stem and root.

In the wood the greatest accumulation is shown by polysaccharides, while the nitrogen fractions increase only at about the same rate as fresh weight (intermediate between total carbohydrate material and water). Asparagine is, if anything, lower than residual N, while protein is definitely below both. Thus there does not seem to be accumulation of any nitrogen fraction in the wood.

#### (d) *Phosphorus.*

Fig. 6 gives the concentrations (per 100 grm. water), the actual weights of total phosphorus per zone, and also the values per 100 grm. fresh weight and per 100 grm. total carbohydrate material. We saw earlier that, whereas nitrogen accumulated mainly in the bark of the two basal stem zones, phosphorus accumulated both in bark and in wood. This storage is followed by depletion from these zones during the last period. It is interesting therefore to note that, while the concentration gradient from stem to root is consistently positive, that along the stem only becomes positive in the later collections when storage in the basal zones is ceasing or being replaced by depletion. Taking total carbohydrate as our reference line we see that, in the root zone, phosphorus is the same at collections 1 and 2 but then falls. In stem zone 1 it increases slightly up to collection 2 and then falls. In stem zone 2 it increases up to collection 3 and then falls. In stem zone 3 it increases for one period, then remains stationary, while in stem zone 4 it increases for the first (only) period observed. On this basis the early period of rapid growth of any zone of bark is marked by phosphorus storage, the mature period of slower

growth by cessation of storage or a depletion. This behaviour will produce changing gradients of storage phosphorus in the main axis so that

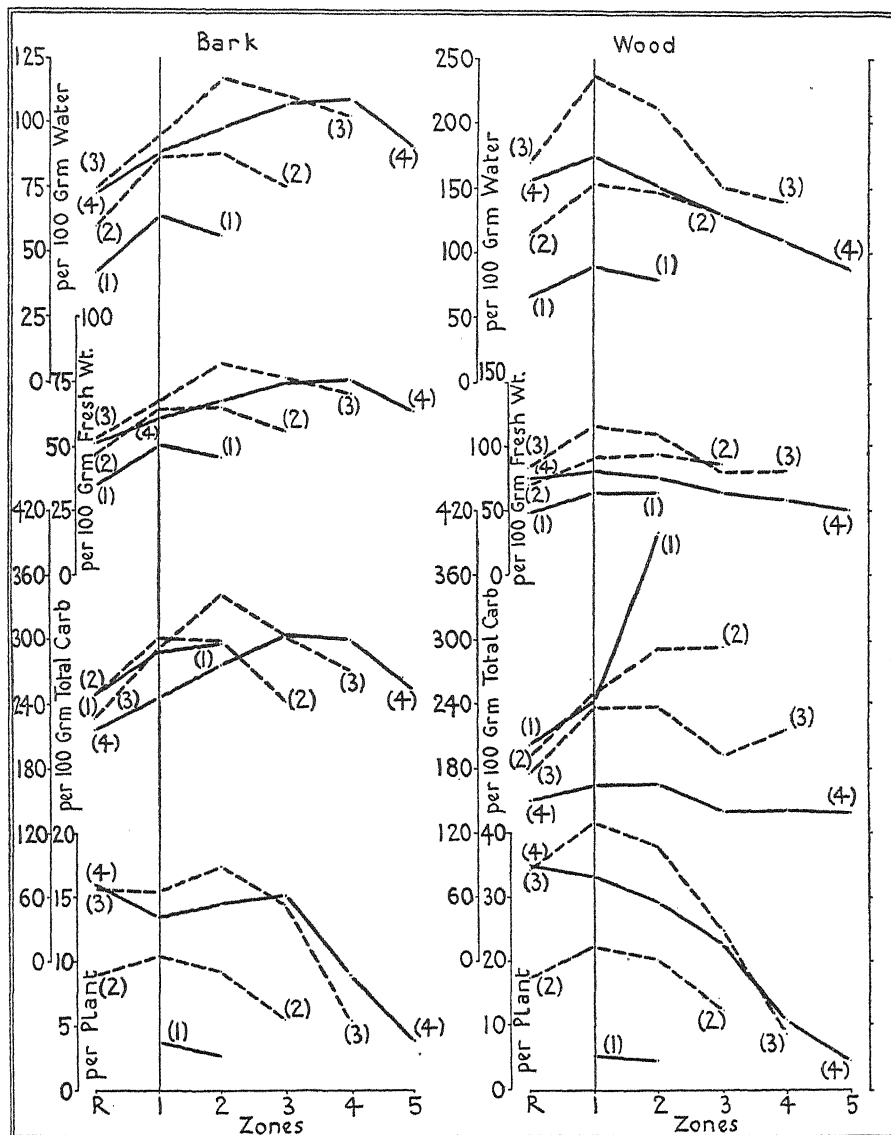


FIG. 6. Weights of phosphorus per zone in bark and wood, and concentrations per 100 gm. water, per 100 gm. fresh weight, and per 100 gm. total carbohydrate material.

even if there is a consistent gradient of mobile phosphorus from the foliage region downwards, the gradients of total phosphorus may be sometimes negative and sometimes positive. We found earlier (8) during periods when there was evidence of downward movement of phosphorus from the

foliage region, sometimes a small negative and sometimes a small positive gradient of total phosphorus in the bark. The present evidence as to storage of phosphorus confirms the suggestion that this might be due to the existence of a negative gradient of storage phosphorus.

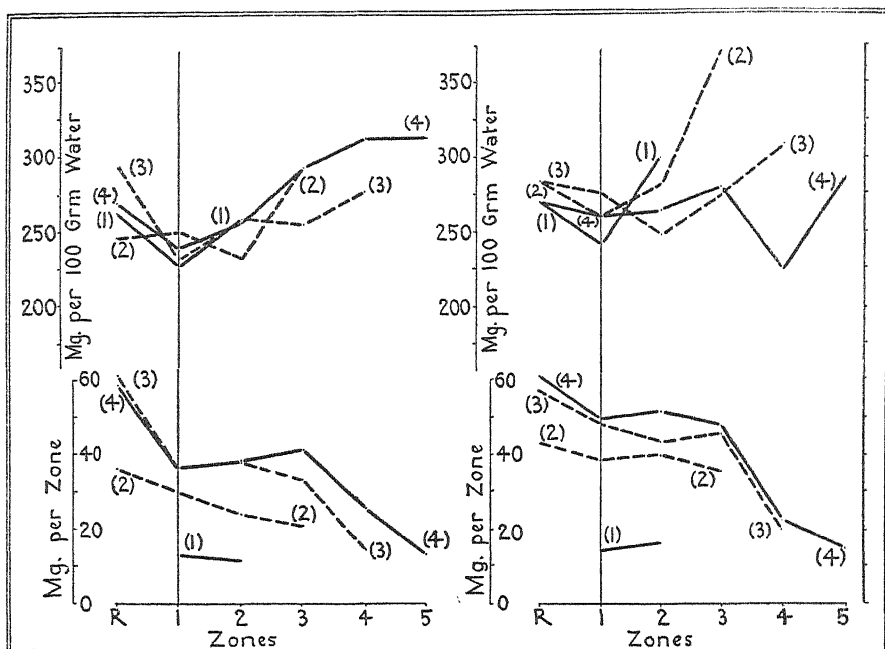


FIG. 7. Weights of potassium per zone in bark and wood, and concentrations per 100 grm. water.

In the wood phosphorus increases more rapidly than fresh weight in the root and two basal stem zones up to collection 3, after which there is a decrease. There is little sign of storage in the upper stem zones and the concentration gradients in the stem (per 100 grm. water) are consistently negative.

#### (e) Potassium.

The weights of total potassium per zone, and the concentration per 100 grm. water, are shown in Fig. 7. Since there is, except perhaps in the root, no marked increase in concentration during development but rather a fall, especially in the wood, no suggestion of accumulation in the older zones of the stem arises, and it is unnecessary to give the values relative to the other reference lines, fresh weight and total carbohydrate material. The actual weight values show that, unlike phosphorus, which in the earlier periods accumulated rapidly and in the last period was depleted, potassium does not decrease during the last period, but may

increase slightly. There is thus no indication except, possibly for the root zone, of storage of potassium in a way that would produce a negative gradient in the bark of the stem. In conformity with this we find the concentration gradients consistently positive in the bark of the stem. In the wood also the gradients are positive but flatten out as development proceeds.

In earlier work we found (8), during periods when there was evidence of downward movement of potassium from the foliage region, negative gradients of total potassium in the bark, and suggested that these might be due to static gradients of immobile or storage potassium. We noted, however, that as the general level of concentration in the bark fell from about 0.4 to 0.24 per cent., the negative gradient also fell to about zero. It seemed probable, therefore, that under conditions of diminished supply there might be no appreciable storage and the vertical gradient in the bark might be positive, representing the positive dynamic gradient with its transport head in the foliage region. In the present experiment that expectation seems to be realized, for the stem gradients are consistently positive and the time changes show there is no storage. In addition, the concentration level for the two basal zones, which correspond to the upper and lower regions of earlier experiments, is about 0.24 per cent., i.e. much below the previous value of 0.4 per cent., which was associated with a definite negative gradient.

(f) *Calcium.*

The concentrations of total calcium (per 100 grm. water), the actual weights per zone, and the values per 100 grm. fresh weight and 100 grm. total carbohydrate are shown in Fig. 8. The amount present increases steadily right up to the last collection, notably in the root. The concentration per 100 grm. water rises both in wood and bark, and we find a definite rise, especially in the wood, in percentage of fresh weight. From collection 2 onwards accumulation of calcium appears to keep pace with accumulation of total carbohydrate material (the highest of our reference lines).

In the earlier paper we suggested that the consistent negative gradients found for calcium might be due to a steady accumulation of this element with age. (In view of the doubt whether the calcium accumulating in the leaf or in the stem can be remobilized we prefer to speak of accumulation rather than of storage, which would imply the possibility of re-utilization.) The present results confirm that suggestion.

(g) *Osmotic Pressure.*

For collections 2 and 3 determinations were made, on the sap expressed from the bark, of freezing-point depression and of electrical conductivity.

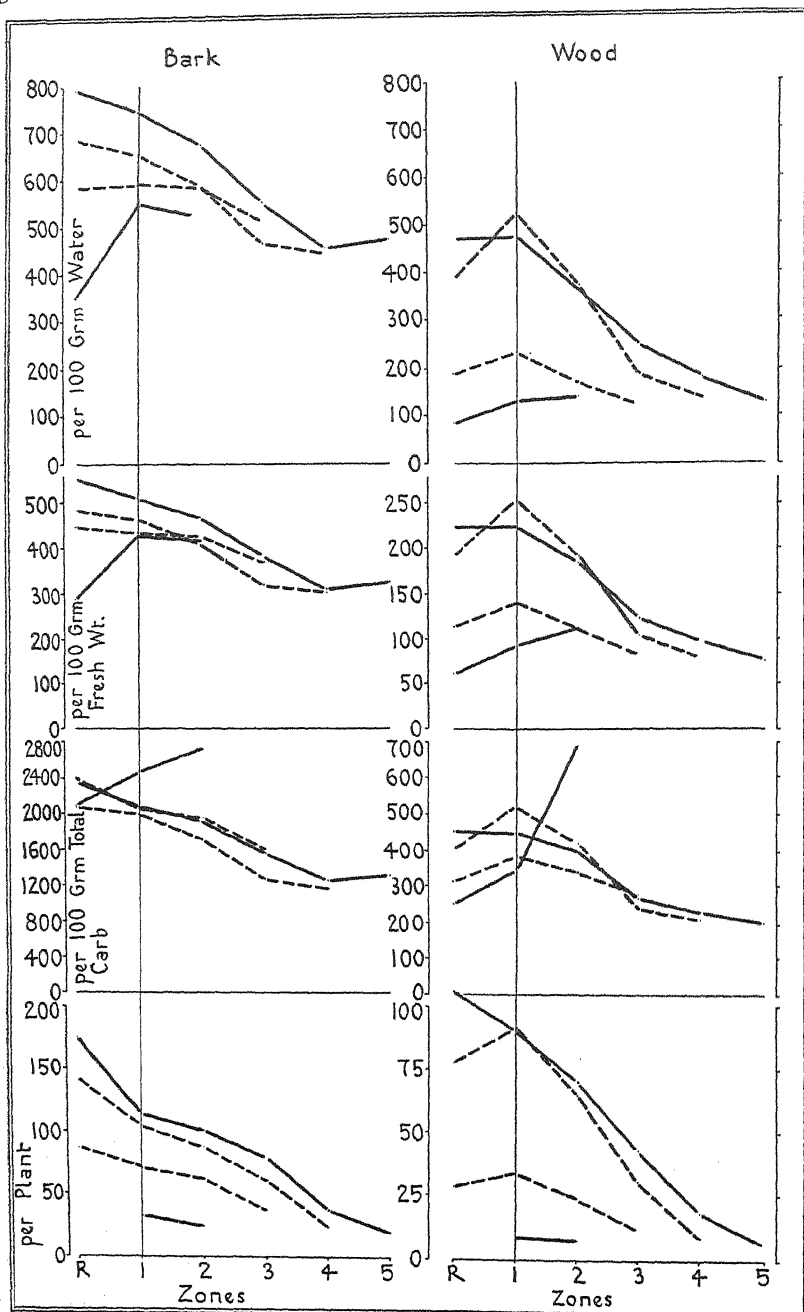


FIG. 8.<sup>1</sup> Weights of calcium per zone in bark and wood, and concentrations per 100 grm. water, per 100 grm. fresh weight, and per 100 grm. total carbohydrate material.

<sup>1</sup> By an oversight the curves for successive collections have not been numbered in the figure. Curves for Collections 1 and 4 (continuous lines) terminate at Zones 2 and 5 respectively. Curves for Collections 2 and 3 (broken lines) terminate at Zones 3 and 4 respectively.



For collection 3 similar observations were made also on the wood sap. The results are shown in Fig. 9.

At collection 2 there is, in the bark, a steep positive gradient of osmotic pressure, but like the gradient of sugars this has very considerably diminished by collection 3. The significance, for transport, of the total osmotic pressure gradient in the phloem will be discussed in greater detail in a later paper dealing more directly with the theory of Münch. Two features of the present data call, however, for some comment here, firstly the magnitude of the gradient observed, and secondly its composition.

At collection 2 the decrements in freezing-point depression from zone 2 to zone 1 and from zone 1 to root zone are  $0.095^\circ$  and  $0.115^\circ$  respectively. The distance between mid points of the zones concerned are 18 cm. and 21 cm. respectively; so that we have osmotic pressure gradients of  $6.3 \left( = \frac{12 \times 0.095}{0.18} \right)$  and  $6.6 \left( = \frac{12 \times 0.115}{0.21} \right)$  atmospheres per metre. This is very much greater than the gradient recorded by Dixon and Atkins (4) for the bark of *Populus*, viz. 1.3 atmospheres in 12 metres, or that found by Harris, Gortner, and Lawrence (5) for leaves inserted at different levels on trees of *Betula* and *Robinia*. It is not, however, much more than would be expected from the vertical gradients of sugars already observed in the bark of cotton (7). These would produce osmotic pressure gradients of 2 to 5 atmospheres per metre. In the bark as a whole the positive gradient due to sugars is offset by a negative gradient due to total crystalloid N but, as we shall see later, other substances may contribute to a positive gradient of osmotic pressure.

In the figure the contribution due to sugars is shown by the curves marked  $\Delta_s$ . At collection 2 this is less steep than the total  $\Delta$  curve, but at collection 3 it is more steep. The contribution,  $\Delta_{s+N}$ , made by sugars and organic nitrogen combined has been estimated by allowing 2 atoms N per molecule for asparagine N, 1 atom per molecule for amino acid N, and  $1\frac{1}{2}$  atoms N per molecule for residual N. The effect of including organic N is, of course, to diminish the positive gradient in the lower regions of the main axis and to make the gradient in the topmost region negative. Subtracting  $\Delta_{s+N}$  from the observed  $\Delta$  there is a residue  $\Delta_r$ , representing the contribution made to osmotic pressure by substances not estimated. At collection 2 this fraction contributes appreciably to the total positive gradient of  $\Delta$  but has a negligible gradient at collection 3. The general parallelism between the curves for this fraction ( $\Delta_r$ ) and for specific conductivity (K) suggests that the residual osmotic pressure is due to electrolytes. Taking the average values for each collection of bark or wood we find that the ratio  $K/\Delta_r$  is 0.0134 and 0.0196 for the bark, collections 2 and 3, and 0.0148 for the wood, collection 3. For values of K, at  $0^\circ \text{C.}$ , of the order  $500 \times 10^{-5}$  the  $K/\Delta$  ratio for uni-univalent salts ranges from 0.011

(Na. acetate) to 0.021 (KCl). For salts with bi- or trivalent ions the ratio would be higher. In view of the fact that correction for viscosity of the expressed sap would increase the observed K values by about 20 per cent.

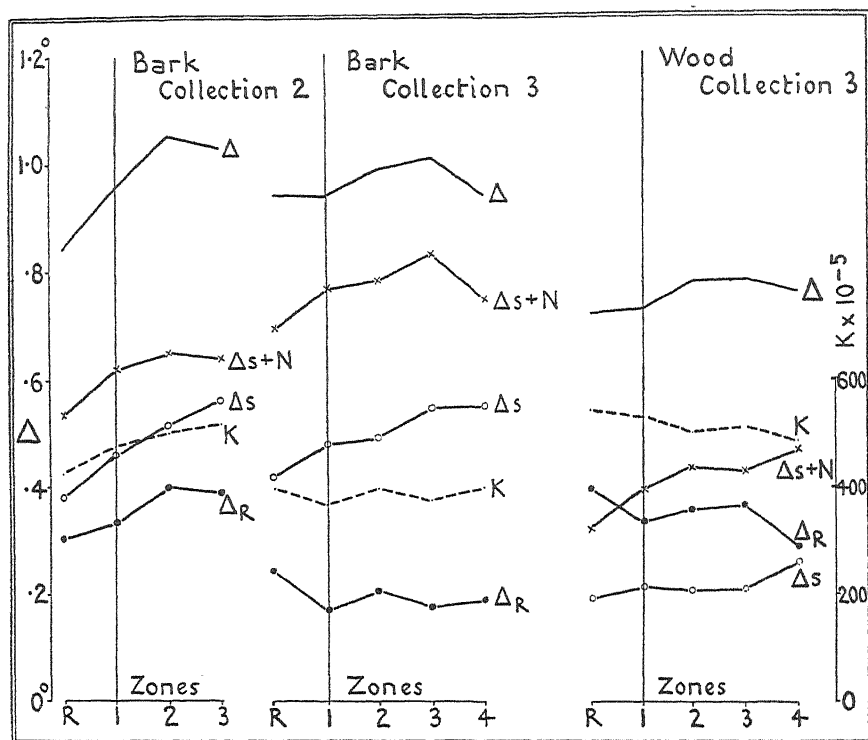


FIG. 9.1 Freezing-point depression and specific conductivity of sap expressed from bark and wood.  $\Delta$  = observed freezing-point depression;  $\Delta_s$  =  $\Delta$  due to sugars;  $\Delta_{s+N}$  =  $\Delta$  due to sugars and soluble organic N fractions;  $\Delta_R$  = residual  $\Delta$  or  $\Delta - \Delta_{s+N}$ ; K = observed specific conductivity at 0°C.

it seems that the  $K/\Delta$  ratio observed is not far from what might be expected if the whole of the residual  $\Delta$  were due to electrolytes. Apparently, therefore, an appreciable part of the total osmotic pressure gradient in the bark of cotton may at times be due to electrolytes. This might be expected from the fact that some ash constituents as well as sugars and organic N are exported from the foliage of cotton and move down the phloem.

The gradients considered here are, of course, gradients in the bark tissues as a whole. In the sieve-tube region the total osmotic pressure gradient should be very much greater, for the sugar gradient is mainly confined to this region, while the negative gradient of crystalloid N is probably due mainly to storage in rays and cortex. Thus Münch's postulate of considerable vertical gradients of osmotic pressure in the conducting

<sup>1</sup> In the figure  $\Delta_s + N$  should be  $\Delta_{s+N}$ .

channels of the phloem, a postulate for which very little direct evidence was available at the time, is confirmed for the cotton plant. It is interesting to note that Dixon's recent observations (3) on the sap exuding from phloem punctures in *Fraxinus excelsior* indicate osmotic pressure gradients, for the sieve-tube sap, of 2 to 9 atmospheres per metre, i.e. values many times as great as those found previously for the whole bark of trees and ranging above those now recorded for the whole bark of cotton. Whether this gradient of osmotic pressure actually produces a mass flow of solution along the sieve tubes is, however, a matter for further discussion.

#### IV. GENERAL DISCUSSION OF THE VERTICAL GRADIENTS OF NUTRIENT ELEMENTS.

The general question under investigation is whether the observed vertical gradients of nitrogen, phosphorus, potassium, and calcium in the bark of cotton are consistent with the view that the downward movement of each from the foliage region is determined by the existence in the phloem of a positive concentration gradient of the mobile form of each. (In the case of calcium the dynamic gradient must be negligibly small, for we could find no evidence of movement.) We had, in general, found negative gradients for all these elements and had suggested that the dynamic gradient of mobile material was being masked by a static gradient of storage or immobile material, the negative sign of this gradient being due to the longer time during which the lower zones of the stem had been accumulating. It seemed possible also that, if supply of any nutrient element were restricted storage might be very much diminished, so that the static gradient would no longer mask the dynamic gradient.

In the present series of observations the ontogeny shows in the bark very marked accumulation of nitrogen, storage of phosphorus followed by depletion, steady accumulation of calcium, but no accumulation of potassium. In conformity with this ontogeny we find consistent negative gradients of total nitrogen and total calcium, and gradients of total phosphorus which are initially negative but become positive later; the total potassium gradients, on the other hand, are consistently positive. In the case of phosphorus and potassium we cannot at present analyse the gradients any further since no fractionation was carried out. There is, however, some evidence from earlier work (8) that where there is a negative gradient of these two elements in the bark as a whole, it is due very largely to the outer half of the bark which consists principally of cortex and ray tissue. In the case of nitrogen the present observations show that the most marked accumulation is that of a crystalloid fraction—the asparagine N. The steep negative gradient resulting from this storage of asparagine almost completely masks a consistent positive gradient in the other main

component of the crystalloid N, namely the residual N. Only in the later periods, when accumulation of asparagine appears to be ceasing, does the total crystalloid N gradient show any signs of becoming positive. This behaviour of asparagine N and residual N respectively is in conformity with what has already been noted (p. 121) as to their distribution in the tissues of the bark.

While much more work must be done before we can definitely identify residual N as the chief form for nitrogen transport along the phloem of cotton, or before we can assert that longitudinal movement of nitrogen is definitely associated with a gradient of this fraction in the direction of movement, it is clear that some at least of the difficulties presented by the observed vertical gradients of crystalloid nitrogen have been removed. In general the working hypothesis, that movement of materials along the phloem is determined independently for each material by the concentration gradient of its mobile form in the channel of transport, would seem to be strengthened by the results of this study.

## V. SUMMARY.

1. The changes, during development, in the amounts of different materials present in successive zones of the main axis of cotton are recorded by observations at monthly intervals.

2. Using three reference lines, namely (i) fresh weight, (ii) water content, (iii) total carbohydrate material, the following conclusions as to storage of different materials during development are reached: (a) Polysaccharides accumulate steadily, especially in the bark; (b) total N accumulates in the bark even more markedly than polysaccharides, but shows little, if any, accumulation in the wood; (c) phosphorus accumulates rapidly in the bark and also in the wood. It shows depletion from the lower zones during the final period; (d) calcium increases in the bark steadily up to the last collection, but only at about the same rate as fresh weight. It shows some accumulation in the wood; (e) potassium shows no sign of accumulation.

3. Where there is evidence of storage of material in the bark, the vertical concentration gradients (per 100 gm. water) are negative. Potassium, which in this experiment shows no sign of storage, has a consistent positive gradient. The results support the view that the observed negative gradients are due to a negative storage component masking a dynamic component of freely mobile material.

4. Storage of nitrogen in the bark takes place very largely as asparagine N and this fraction is responsible for the observed negative gradient of crystalloid N. Residual N, on the other hand, maintains a consistent positive gradient and may represent the mobile component.

5. The vertical gradients of sugar concentration and of total osmotic pressure in the bark are steep in the early stages when growth is more rapid, but flatten out as development proceeds. Electrolytes may contribute to the total positive osmotic pressure gradient.

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# The Electric Charge of the Colloid Particles of Protoplasm.

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With Plate I and one Figure in the Text.

THE electric charge of particles in colloidal suspension is best detected and measured by cataphoresis, that is, by observing their migration when an electric current is passed through the solution. If the particles are positively charged they migrate towards the cathode, if negatively charged, towards the anode. Heilbrunn (7) in his monograph brings out clearly: (i) the importance of the electric charge of the colloidal particles of protoplasm; (ii) the difficulties involved in cataphoresis experiments with living protoplasm; and (iii) the caution that should be used in interpreting the results of observation.

Since Bois-Reymond (14), observed the starch grains in the living cells of a potato tuber migrate towards the anode, numerous investigators have studied the effect of the electric current on the movement of the contents of living cells, alike plant and animal. Jürgensen (8), Kühne (9), Velten (19), Verworn (20), and Taylor (17) observed directly under the microscope the movement of the cell contents due to the passage of an electric current, and McClendon (10), Hardy (6), and Meier (11) passed an electric current through a tissue and, fixing it immediately, observed in the sections the disposition of the cell contents. The conclusions of these investigators are, however, not in accord. The observations of Kühne on myxomycete plasmodium, of Verworn on amoeba, of Hardy on onion-root tip, would indicate that the particles of protoplasm are *positively* charged; while those of Kühne on *Tradescantia* hair, of Jürgensen on *Vallisneria*, of McClendon on hyacinth root, of Meier on pea root-tip, of Taylor on myxomycetes, would indicate that the particles of protoplasm are *negatively* charged; those of Velten with low current intensity on streaming plant protoplasm and of Verworn on *Aethalium septicum* would indicate nothing about the nature of the electric charge, since they found that the particles of protoplasm would move either to the anode or to the cathode if they

migrated at all. In view of conflicting results, this problem has been investigated afresh. (For a comprehensive survey of the existing literature, see Heilbrunn's monograph.)

#### THE MATERIAL.

It is obvious that the cataphoretic response of the particles of protoplasm in a living cell is best studied when a single cell is subjected to a uniform electric field and the migration of the particles is observed directly under the microscope. Gaidukov (4) and Price (13) have described the advantages of the dark-ground illumination for observing the movements of the protoplasmic suspension. But the use of the dark-field limits the choice of material. The cell has to be either a unicellular organism or a cell of a tissue one cell thick; the outer membrane or the cell wall must be optically homogeneous; the diameter of the cell must not be so small that the diffraction image of the wall interferes with the observation of the cell contents; and the cell must not contain a large number of bodies which scatter light.

In 1930 Professor Robert Chambers and I tried to determine the electric charge of the protoplasmic granules of *Amoeba dubia* and *A. proteus* in his New York laboratory. On account of the natural movement of the granules, we could come to no conclusion from our cataphoresis experiments with these animals. I have since tried other protozoa, *Holomastigotoides* from termites and *Opalina*, *Nyctotherus*, and *Balantidium* from toads and frogs, but found them equally unsuitable for cataphoresis experiments.

A mature plant cell offers one special advantage in that the entire cell contents is enclosed in the inelastic cell wall. Several plant hairs from petioles of *Urtica dioica* (stinging nettle), of *Cucurbita pepo*, and of *Ocimum sanctum* have been examined, but these, on account of the streaming movement of their protoplasm, are not the best materials for cataphoresis experiments. Only the apical end of the *Urtica* hair, which is filled with the protoplasmic granules, can be used for observing the cataphoretic response of the granules, since the velocity of their streaming movement is considerably less at the apical end than in the rest of the cell.

For microscopic observation of the cataphoretic migration of the particles of protoplasm in a single living cell, the electrodes of necessity have to be very near the ends of the cell, and since protoplasm is at once a very sensitive and complex material the complications due to electrolysis and other secondary changes have to be taken into account. These disturbances can be minimized by (i) using a low intensity of current for a short period, and by (ii) observing the migration of the particles equidistant from the electrodes (see 18) immediately the current is switched on, for the magnitude of the electrode disturbances will be proportional to the



intensity and duration of the current, and the cataphoretic response of colloid particles in suspension is practically instantaneous. Cotton and Mouton (2) have shown that the colloid particles can easily follow an alternation of the direction of a current exceeding 1,000 per second. But to observe the instantaneous cataphoretic response in a living cell there should be no autonomous movement of the protoplasm apart from the Brownian movement of the particles.

The root-hairs of the water fern *Azolla pinnata*, which I first found quite accidentally in my *Nitella* supply, seem to be an ideal material for cataphoresis experiments with living protoplasm. The many advantages of *Azolla* are: (i) it grows in great abundance throughout the year in fresh water; (ii) the number of hairs in a single root of *Azolla* varies from 400 to 700 and thus comparable material for several experiments is available; (iii) the root hairs (always single cells) can be easily isolated under water, their natural environment, and kept alive for twenty-four hours under suitable mounting; (iv) the length of individual root hairs varies from 0.5 mm. to 2.5 mm., and the diameter from  $16\ \mu$  to  $25\ \mu$ , so the root hairs are very suitable for observations in the dark-field; and (v), except for a few  $\mu$ s at the tip and at the base, an *Azolla* root hair is practically a cellulose capillary of uniform diameter filled with transparent protoplasm in which the only natural movement that can be observed is the Brownian movement of the protoplasmic suspension.

#### THE EXPERIMENTAL ARRANGEMENT.

The general experimental arrangement is similar to the arrangement that has already been described (16). Plant hairs are isolated by teasing under water with a needle sharpened at the end to a knife edge. Isolated hairs are then transferred to a drop of water on a very clean, fat-free coverslip and, after removal of any excess water with a micro-pipette, are covered, together with their surrounding thin film of water, with liquid paraffin (nujol) to prevent evaporation. The coverslip is then inverted with a sharp twist and placed over the chamber of the microscope. *Urtica* hair thus mounted will continue to show the streaming movement of the protoplasm for more than three days, and the root hairs of *Azolla* the Brownian movement of the colloid particles of protoplasm for more than twenty-four hours.

Leitz's cardioid condenser, which can be used with Chambers's micro-manipulators, was employed for the dark-field observations. For microscope illumination a 400 c.p. projection lamp was used for visual observations and a Leitz's small arc lamp for micro-photographs. A litre flask containing alum solution was placed in the path of the light to cut off the heat rays as far as possible.

The electric connexions with the hair were made by bringing up the

electrodes with the help of the micro-manipulators to the surrounding film of water. The electrodes were connected in series with a sensitive galvanometer and an accumulator. A tapping key was used for switching the current on and off, and a mercury reversing key for changing the direction of the current. The galvanometer was kept short-circuited when not required for measuring the intensity of the current flowing through the film of water surrounding the hair. From this measurement, of course, no idea could be formed as to the current which actually flowed through the protoplasm of the cell.

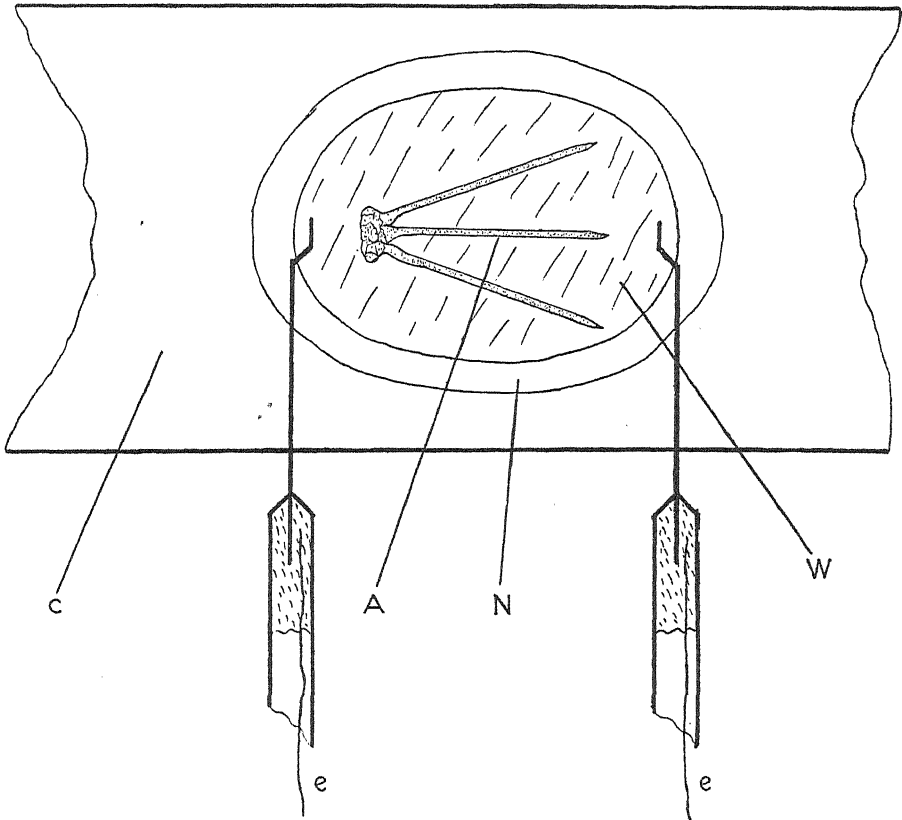
Preliminary experiments showed that all types of electrodes—platinum, platinum-iridium, silver-silver chloride, and agar electrodes of the Gelfan (5) type—gave exactly similar results. Since platinized glass electrodes (15) could be easily made, and particularly of any required shape and size of contact surface, those of the shape as in the text-figure were chiefly used.

#### EXPERIMENTS.

Observations were taken ten to fifteen minutes after the hair was mounted in a hanging-drop preparation. The portion of the hair to be observed was first brought in the field of the high-power objective, and then, under the lowest magnification, the electrodes were pushed up into the surrounding film of water, as far away from the ends of the cell as possible, and in appropriate positions to subject the entire length of the hair to a uniform electric field (see Text-fig.). To avoid unnecessary heating, the light was kept on only for the period of observation. With the particles of protoplasm of the hair in the focus of the high-power objective the current was switched on and off, and also its direction was reversed, and the consequent cataphoretic response of the particles of protoplasm was simultaneously observed.

*Observations with the petiole hair of Urtica dioica: Experiment 1.*—Normal microscope illumination (transmitted light) was chiefly used for these observations. As already stated, only in the apical end of a hair of *Urtica dioica* can one observe the cataphoretic response of its protoplasmic granules. Over fifty hairs were examined with different types of electrodes. It was invariably found that when the electrode near the tip of the hair was made the cathode, the particles of protoplasm moved away from the tip-end, and that at the break of the current the particles migrated back to the tip. When the current was reversed, i.e., when the electrode near the tip was made the anode, the protoplasmic granules accumulated at the tip. On account of the natural streaming movement of the protoplasm of the hair, these effects were not very striking. The electric field to which the hair was subjected varied from 4 to 40 volts per cm., but in every case the immediate response of the granules was the same, i.e., they moved towards the anode and away from the cathode, irrespective of the intensity of the

current. It was found that, when the intensity of the current flowing through the water film surrounding the hair did not exceed 15 microamperes and its duration was not more than 5 seconds, the cataphoretic response of the protoplasmic granules of the *Urtica* hair could be observed



TEXT-FIG. Diagram of experimental arrangement as seen from above. C. coverslip; A. *Azolla* root hair; W. surrounding film of water; N. paraffin film; e, e'. electrodes.

at least a dozen times in the same hair. With higher intensities and prolonged duration of the current the protoplasm is injured and coagulates towards the tip if the adjacent electrode happens to be the anode, and away from it if the cathode. Only at the *break* of currents of low intensities could the reverse movement of the granules be observed.

*Observations with the root hair of Azolla pinnata: Experiment 2.*—The protoplasm of the root hair of *Azolla* is very transparent, and its particles are difficult to distinguish under normal microscope illumination, therefore all observations with *Azolla* root hair were made under the dark-field. The protoplasmic particles of the root hair in the dark-field appear as bright dots and dashes in vigorous Brownian movement. The tip of the

hair can be used for photomicrography of the cataphoretic displacement of the particles of living protoplasm, and one can measure the velocity of single particles in the middle portion of the hair (Plate I, Fig. 1). Out of the several hairs in a preparation (Text-fig.) only those without any sign of injury were used for experimental observation. The cataphoretic response of the protoplasmic particles, described in the previous experiment, was observed more strikingly in the root hairs of *Azolla*, i.e., the protoplasmic particles migrated towards that end of the cell whose adjacent electrode was made the anode. The micro-photographs given in Plate I bring out clearly that (i) when the electrode adjacent to the tip of the hair was made the cathode the cytoplasmic particles moved away from it; (ii) at the 'break' of the cathode the particles moved back in the reverse direction; (iii) at the 'make' of the anode the protoplasmic particles moved towards the tip; (iv) at the 'break' of the anode the particles moved away; and lastly (v) in course of the experimental conditions the protoplasm of the root hair was not injured, since the same root hair could be used to observe the cataphoretic responses of the cytoplasmic particles several times, in spite of the fact that a rather high intensity (20 micro-amperes) was used to secure photographs.

*Cataphoretic velocity of different cytoplasmic particles of Azolla root hair: Experiment 3.*—The middle portion of the *Azolla* root hair (see Plate I, Fig. 1), where the cytoplasmic particles would be fairly equidistant from the electrodes, was used for the measurement of the cataphoretic velocity of the particles. In view of the fact that the root hair is cylindrical, no accurate optical section was possible, particularly in the dark-field. The microscope was focused at the maximum breadth of the cell. The velocity of single particles was observed by noting the time it took to cover a definite number of the scale divisions of the micrometer eye-piece immediately the current was switched on. When the intensity of the current flowing through the surrounding film of water was low (less than 10 micro-amperes) and its duration not more than 5 seconds, several observations could be taken on the same hair. As has already been stated, no definite idea can be obtained of the potential gradient to which the protoplasm inside the hair is subjected, and therefore the actual cataphoretic velocity of the protoplasmic particles per volt/cm. per second could not be determined. But the velocity of the different particles of the same hair when subjected to varying potential differences between the electrodes was observed. More than fifty root hairs of *Azolla* were examined, and in every one it was found that (i) the particles very near the cell walls scarcely moved, and, if at all, towards the cathode; (ii) particles farther away from the cell walls—which alone would show true cataphoretic migrations (see 12)—moved towards that end of the cell adjacent to the anode; (iii) immediately at the 'break' of the current there was a reverse movement of

the particles; (iv) the particles which appeared to be about midway between the cell walls (accurate determination of the position of the particles was impossible) showed the maximum velocity, irrespective of their shape or size; and (v) the velocity of the particles of a hair increased proportionately with the increase of potential between the electrodes. The velocity of migration of the different particles in the same hair was found to be very uniform for several determinations including reversal of the direction of the current. Table I gives the details of a typical observation on a single root hair.

TABLE I.

*Cataphoretic Velocity of the Protoplasmic Particles of a Single Root Hair of Azolla.*

(P) approximate diameter of the particle; (V) volts between electrodes; (S) distance covered in time (t); (c.v.) calculated velocity of particles per second; R, with direction of the current reversed. Length of hair = 1.67 mm., diameter = 22  $\mu$ .

(P.) in $\mu$ .	(V.) volts.	(S.) cm. $\times 10^{-4}$ .	(t.) secs.	(c.v.) cm. $\times 10^{-4}$ .
0.5	2	26	5	5.2
2.0	2 R	16	3	5.3
1.0	2	16	3	5.3
2.0	2 R	22	4	5.5
0.5	4	32	3	10.6
2.0	4 R	21	2	10.5

## CONCLUSION.

The streaming granules of the *Urtica* hair are protoplasmic particles, and so also are the particles in the root hairs of *Azolla*, the cataphoretic migration of which has been observed in the experiments described above. That the particles of protoplasm are electrically charged is a well established fact (see 7). The Brownian movement of the particles of *Azolla* root hair shows that they are freely suspended in the dispersion medium of the protoplasm, and have no autonomous movement. The observed migration of the particles lying about midway between the cell walls which is induced by the passage of an electric current is thus due to cataphoresis. That the different protoplasmic particles of varying shapes and sizes showed the same cataphoretic velocity is in accord with von Smoluchowski's formula for cataphoresis, which has been experimentally verified by Abramson and Michaelis (1). They found that in dilute protein solutions (a) the velocity of cataphoresis did not depend to any measurable extent on the bulk conductivity of the suspended particles, and (b) globules of paraffin oil or mastic 1  $\mu$  to 5  $\mu$  in diameter, needles of asbestos from just visible lengths to 70  $\mu$ , and needles of m-aminobenzoic acid crystals up to 100  $\mu$  long showed, within the limits of the experimental error, the same cataphoretic velocity.

It has been shown that as long as the current continues the protoplasmic particles of different shapes and sizes visible under the dark-field move towards the anode, and that reverse movement of the particles takes place immediately at the break of the current, when the intensity and duration of the current is not sufficient to injure the protoplasm. With high intensity of current the protoplasm coagulates at the anodic end of the cell. From the results of the experiments the conclusion must be drawn that the protoplasmic particles, at least of the petiole hairs of *U. dioica* and of the root hair of *A. pinnata*, are *negatively* charged, and so far as the changes induced in a living cell by the migration of its protoplasmic particles are concerned, the effect at the 'break' at the anode is similar to that at the 'make' at the cathode.

#### SUMMARY.

The cataphoretic migration of the particles of living protoplasm in the single cells of the petiole hair of *Urtica dioica* and of the root hair of *Azolla pinnata* has been observed in dark-field with a hanging-drop preparation.

These observations show that (i) the particles of protoplasm carry a *negative* charge, since their cataphoretic migration is always towards that end of the cell whose adjacent electrode is made the anode; (ii) immediately at the 'break' of the current the particles migrate in the opposite direction; (iii) when the intensity of the current is not such as to injure the protoplasm, these observations can be repeated several times on the same hair with reversal of the direction of current. Further, the velocity of cataphoretic migration of single particles of protoplasm of the *Azolla* root hair has been measured, and this velocity has been found to be independent of the shape and size of the particles.

I take this opportunity to acknowledge the generous financial help, from which the expense of this investigation has been met, received from Mr. and Mrs. L. K. Elmhirst and Mr. and Mrs. H. Crowley. I am indebted to Prof. L. V. Heilbrunn for valuable suggestions, to Prof. A. V. Hill for his criticism of this paper, and to Mr. S. M. Sircar, Research Scholar of this Laboratory, for helpful assistance with some of the observations.

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## EXPLANATION OF PLATE I.

Illustrating Mr. B. Sen's paper on 'The Electric Charge of the Colloid Particles of Protoplasm'.

All the figures are dark-field photomicrographs of the root hair of *Azolla pinnata*.

Fig. 1. Middle portion of root hair where the cataphoretic velocity of the particles is most clearly observed.

Fig. 2. Tip of root hair.

Figs. 3-5. Cataphoretic effect with cathode near tip of root hair.

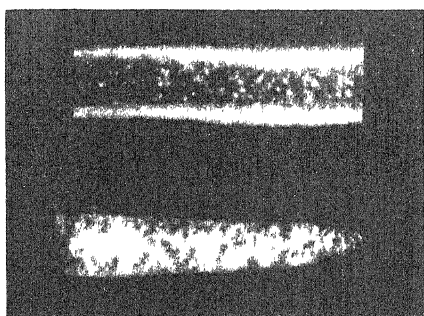
Figs. 6-8. Similar to Figs. 3-5, but immediately at break at cathode.

Figs. 9 and 10. Immediately at 'make' at anode.

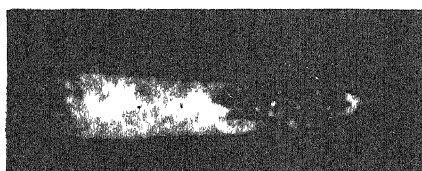
Fig. 11 and 12. Immediately at 'break' at anode.



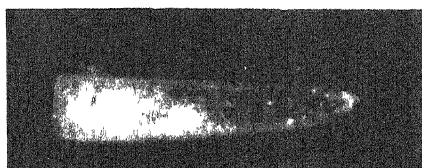




2



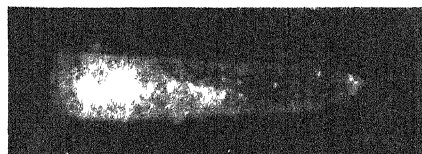
3



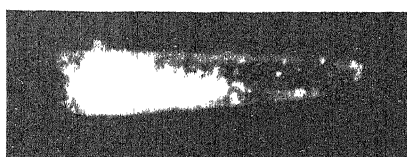
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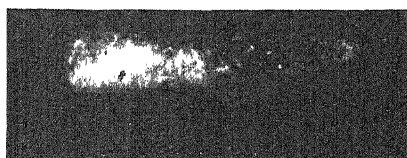
5



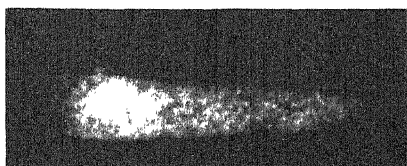
6



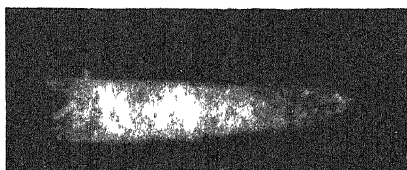
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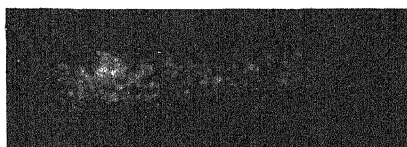
8



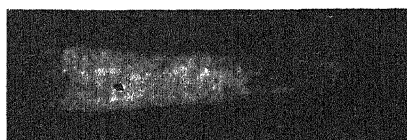
9



10



11



12

Huth coll

SEN — ELECTRIC CHARGE OF COLLOID PARTICLES OF PROTOPLASM.



# Meiosis and Catenation in Certain Crosses of *Oenothera rubricalyx*.<sup>1</sup>

BY

M. VERBRUGGE, B.Sc.

With Plate II and three Figures in the Text.

## INTRODUCTION.

THIS work was primarily undertaken to determine the catenation in certain crosses of *O. rubricalyx*. The material was collected during August of 1931 from plants grown by Prof. Gates in Regent's Park. I wish to thank Miss K. Goodwin and Mr. D. G. Catcheside for allowing me to use some of the material which they collected.

The buds were collected only between 11 a.m. and 1 p.m. (summer time) on fairly bright days, as this was found to be the time during which diakinesis was taking place. Whenever possible, plants which had not begun to flower were chosen, buds of all sizes being removed in order, from those containing pollen to small scarcely differentiated ones. After removal of the sepals and petals the bud was dipped into Carnoy's fluid for about 30 seconds, to dissolve the waxy excrescences, and then rinsed in water. The tops of the anthers and the ovary were cut off with a pair of sharp scissors, to allow more rapid penetration, and the bud was dropped into a bottle of fixative, which was then attached to the exhaust pump. Flemming's weak solution was perhaps best, though the medium solution also gave good results. The fixative 2B of La Cour (23) was not at all suitable for this material, as it caused a great deal of shrinkage and distortion of the cells.

For embedding, the chloroform method recommended by La Cour (23) was used. Most of the sections were cut  $14\mu$  in thickness, as this gave a good proportion of uncut nuclei without interfering with the stain. Sections were stained with the gentian violet and iodine, and the gentian violet, iodine, and chromic acid methods of La Cour (23); both gave satisfactory results.

<sup>1</sup> Thesis approved for the degree of Master of Science in the University of London.

## PART I.

*Stages before diakinesis.*

As the pollen mother-cells grow they become rounded and seem to press out the tapetal cells, since these become rather irregularly arranged. Between the pollen mother-cells can be seen small spaces at the angles. As the pollen mother-cells continue their growth they become more regular in shape, with more spaces between them. The wall thickens and the cytoplasm becomes more granular, while the nucleus is now nearly spherical. It is also very much larger, having grown comparatively much more than the cytoplasm. The nuclear membrane is extremely thin and the nucleolus sometimes appears to be firmly pressed against it, and is then lens-shaped. The threads, which are much thicker and shorter, only touch the membrane at intervals. Sometimes they have the beaded appearance which is often seen in threads at this stage (Pl. II, Fig. 2). The tapetal cells are again seen to be larger, and they are now binucleate.

Following this, the cells are more rounded off, while the wall is slightly thicker. The nucleus is smaller than before, and is seen to contain threads which are still shorter and thicker. The nucleolus usually stains more faintly, but in some material of *O. rubricalyx*  $\times$  *O. blandina* the endonucleolus described by Cleland (3), Sheffield (36), Kulkarni (22), and others, is visible as a small darkly stained dot. Sometimes the thread seems to be connected to the nucleolus by means of the endonucleolus, but this was not found in many cases. In one pollen mother-cell a nucleolus was seen in which the endonucleolus appears to be divided (Pl. II, Fig. 4). In the material of *O. deserens*  $\times$  *O. rubricalyx* the endonucleolus was not seen, but the nucleoli were as darkly staining as the thread.

Pl. II, Fig. 3, of *O. rubricalyx*  $\times$  *O. blandina* shows a nucleus with a very much tangled thread. On careful examination it was possible to see that along the threads were considerably thinner portions which apparently represent the connexions between the chromosomes. I, II, show a small complete ring which appears to be made up of two chromosomes. III, IV, V, seem to be three chromosomes which form part of a ring or chain, the rest of which could not be followed in the tangled mass of threads. Chromosome IV is seen to touch the membrane near the middle of its length. (The chromosomes have been drawn slightly thicker than they appear, to make the figure clearer.)

In the stage which follows, the threads are still shorter and thicker, and the nucleolus is usually very pale. It was extremely difficult to trace the threads along their whole length, but it was often possible at this stage to distinguish one or two separate rings. Pl. II, Fig. 5, of *O. deserens*  $\times$  *O. rubricalyx* (in which the catenation is a ring of four chromosomes and five ring

pairs) shows a nucleus in which it was possible to make out five small rings, I, II, III, IV, V, and one larger ring VI.

The threads continue to condense and are now seen to be definitely made up of chromosomes. Usually they are rather tangled, but in several places they radiate out towards the periphery (Pl. II, Fig. 6), and the nucleolus is still present.

### *Diakinesis.*

The pollen mother-cells are rounded off, fairly thick walled, and contain coarsely granular cytoplasm, in the centre of which is the spherical nucleus. The membrane is now so thin that it sometimes only becomes visible after very careful focusing. The nucleolus is present, but it is usually very pale. In several nuclei more than one nucleolus could be seen (Pl. II, Fig. 10). At this stage the catenation can be determined fairly easily, the chromosomes are fairly regular in outline, and similar in shape and size, and are definitely arranged in groups. Often the pairs are interlocked with each other or with the ring. Pl. II, Fig. 9, shows all the pairs interlocked with the ring. The configuration varies in the different forms, and these will be dealt with in Part II.

### *Later stages.*

The characteristic zigzag arrangement of the chromosomes can be seen in a large number of pollen mother-cells. Pl. II, Fig. 12, shows the beginning of the zigzag orientation within the nucleus, and Pl. II, Figs. 19, 20, show a later stage. Sometimes it is seen that two neighbouring chromosomes are moving in the same direction, so that non-disjunction will result. Pl. II, Fig. 16, shows some of the connexions broken, and along the fibres can be seen thickenings of chromatic material, so that it appears that some active force must be pulling the chromosomes apart and towards the poles.

The telophase stages and the homotypic division correspond to those described by many observers.

### INTERPRETATION.

In the early development of the merismatic archesporium there is apparently a rapid rush of food and an increase of turgidity which cause the pollen mother-cells to increase in size, and to become rounded off. The pollen mother-cells in enlarging appear to exert considerable pressure, for the tapetum becomes very irregular, and cells outside it are often crushed. At first the most marked increase is in the cytoplasm, and although the nucleus enlarges slightly it appears to meet with resistance from the cytoplasm, for it ceases to be spherical and is considerably affected by the shape of the cell itself. The nucleolus too is affected, and travels to the narrowest part of the nucleus where the inward pressure is

smallest. The nucleus then increases considerably in size at the expense of the cytoplasm. At the same time the wall of the cell becomes thicker. With the influx of food into the nucleus one may expect an increase of internal pressure and the nucleus again becomes spherical, while the nucleolus is often pressed against the membrane. The increase in size of the nucleus causes the membrane to become stretched, for it gradually becomes thinner and thinner. The network seems to be stretched out too, and pressed against the membrane. It does not grow at the same rate as the nucleus and so becomes partly detached from the membrane. As growth proceeds the thick portions are seen to disappear and the network gradually becomes more uniform, and appears as a tangled mass of threads. The large number of threads makes it seem likely that the appearance of a network is, in part at least, due to the crossing of threads. The disappearance of some of these threads may be due to breaking or dissolution. Martens (31, 32) has, in living material, shown that the network of mitotic nuclei is composed of zigzag chromosomes connected to each other by anastomoses. As the nucleus increases the network becomes stretched, the chromosomes straighten out, while the connexions gradually break and disappear, so that the number of threads is reduced. In the material under examination too, the network seems to be made up of a number of entangled threads connected by anastomoses. As the nucleus increases in size the threads become drawn out and the breaking of the connexions results in a reduction in the number of threads. Towards the outside of the nucleus the breaking should be most evident. In this connexion it is interesting to find that the beaded appearance sometimes shown by the thread (Pl. II, Fig. 2) is most noticeable towards the periphery of the nucleus. A figure (Pl. XVI, Fig. 5) in a paper by Gates and Goodwin (14) shows this very clearly. In mitosis in *Narcissus*, Hedayetullah (18) has shown that the chromosomes consist of two parallel threads which during prophase appear as strings of chromomeres. By careful examination he found that the anastomoses between the threads connected adjacent granules. In *Oenothera* it is very difficult to study chromosome structure, but the beaded appearance suggests that the chromosomes are, here too, made up of strings of granules, which only become evident when they are thickened by material from the anastomoses.

Not only the length but the thickness of the threads alters, and gradually it is possible to see that the chromosomes are joined end to end (Pl. II, Fig. 3). It is not until the looped stage, however, that it is possible to see clearly the arrangement of the chromosomes, and at this stage it has been shown that the chromosomes are arranged in rings (Pl. II, Fig. 6), which give the characteristic configuration at diakinesis (Gates and Goodwin (14)).

*The darkly staining bodies in the reticulum.*

The presence of these darkly staining bodies has not often been disputed, but their significance has been much discussed. Miss Leliveld (25) describes fourteen such bodies in *Oenothera*, and she regards them as the prochromosomes or chromocentra. On the other hand, Gates and Goodwin (14) have shown that there is great variety, both in size and number, of these bodies, so that they do not attribute any special function to them. In the material examined it was found that there was a great deal of variety in size and number (Pl. II, Fig. 1), and that many of these bodies appeared to be in contact with the membrane. As the network became stretched it was seen that some of these bodies disappeared altogether, so that it seems that their appearance may have been due to several threads crossing at one point. On the other hand, some of them seem to persist, and to elongate slightly as the threads become stretched. Possibly these bodies may represent the point of attachment of the chromosomes, and since in *Oenothera* this point is centrally placed the name 'chromocentra' seems very suitable. It is interesting to find that Koerperich (21) has shown that in mitosis the chromosomes are always in contact with the cytoplasm at a certain point.

When the chromosomes are fully formed they have an attachment constriction near the middle, so that there is definitely a central point of the chromosome which differs from the rest. Probably this point is less elastic than the rest of the chromosome, so that when this is thick it appears as a constriction, and when the chromosome is spun out, as in resting stages and prophase, it appears as a thickening.

The darkly staining bodies in the reticulum would seem to be of two kinds, those having a definite significance, the chromocentra, which represent the point of attachment of the chromosome, and those which are merely the optical result of a tangled mass of threads.

*Synizesis.*

Synizesis was seen in much of the material examined, but many of the pollen mother-cells in which it appeared were slightly plasmolysed, so that it may sometimes have been an artifact. In some of the anthers there were loculi in which the lower pollen mother-cells were in an early spireme stage, while the upper ones were in metaphase, but none showed synizesis. In all the material examined it was evident that division began at the top of the loculus. If synizesis did take place in these pollen mother-cells it is likely that it would be seen. This kind of evidence, and that obtained by many workers on the action of fixatives, suggest that synizesis is really an artifact.

On the other hand, it is impossible to neglect much careful work which

has been done to show that this stage is present in well-fixed material, and even in living material (Sargant (34)). Some consideration of the factors which might bring it about should lead to a satisfactory explanation. The thread touches the nuclear membrane in several points, and it is not unlikely that a change of conditions might cause some alteration in the nature of the membrane which might lead to a breaking of the contact, and a total collapse of the thread. Not only fixatives could bring about such a state, but possibly changes in such conditions as temperature, moisture, and food supply, which affect the plant in the living state.

The simplest explanation seems to be that synizesis is not an essential stage in meiosis, and that its frequency is due to the fact that it may readily be brought about by both natural and artificial factors.

#### *Continuity of the spireme.*

Most workers on *Oenothera* agree that at no time during prophase is it possible to see free ends of the threads in uncut nuclei. This might, of course, favour the idea that the spireme is continuous (Illick (20)), but recent evidence shows that at no stage is there a single continuous spireme, except in forms having a ring of fourteen chromosomes. At diakinesis a definite number of rings, generally closed, can be seen. There is now sufficient evidence to show that there are several spiremes present from an early stage, and, as it is nearly impossible to follow the threads in the earliest stages it is necessary to deduce the presence or absence of a continuous spireme from theoretical considerations. The absence of free ends points to the probability of the chromosomes never being free from each other during heterotypic prophase, and it therefore seems quite possible that they become joined in definite groups in the previous telophase. Perhaps a study of the premeiotic telophase stages may give some practical data bearing on this point.

#### *The thickening of the thread and the nucleolus.*

During early prophase a very thin thread is present, and this is gradually seen to shorten and thicken as meiosis proceeds. As it is evident that the chromatic material increases, it is necessary to have some further explanation for the appearance of more chromatin. The way in which this addition is made, and the source of the material, are extremely difficult problems which have been approached in a number of ways. Fikry (9) gives a full account of various points of view and he shows the difficulties which lie in the way of accepting any of the views put forward. Here only the most widely held views will be considered.

The part played by the nucleolus and the method of addition of chromatin appear to be closely related problems. The nucleolus may contribute directly or indirectly to the formation of the chromosomes, or it may be an ergastic substance of no definite importance. If the nucleolus



contributes directly to the formation of the chromosomes there must be connexion between the nucleolus and the threads. Such connexion was first observed by Farmer (7) and it has since been demonstrated by Lenoir (27, 28), Latter (24), Sheffield (36), Zirkle (39), and others. There is the possibility then that material may flow along the spireme from the nucleolus, but it may do this by flowing inside the spireme, as through a tube, as Lenoir (27-29), and Zirkle (39) suggest, or outside the thread as Latter (24) maintains. In the material examined many cases of direct connexion between the nucleolus and the threads were observed. On the other hand, this was by no means a universal phenomenon, and this, coupled with the fact that there is no continuous spireme (at least during late prophase), would exclude such an explanation.

The nucleolus may therefore contribute indirectly to the thickening of the threads, or it may be of no importance in this connexion. Earlier cytologists suggested that the nucleolus consisted of chromatin which was gradually transferred to the chromosomes as they developed, but by microchemical tests Zacharias (38) was able to show that this was not so, and his results have been confirmed by those of other workers (Yamaha and Sinotô, 37; Zirkle, 39), and by those of von Schustow (35) obtained by ultra-violet illumination.

As early as 1907 Gates suggested that the nucleolus was composed of two substances, and there is growing evidence that this is the case. It has, however, been maintained that there is no diminution in the size of the nucleolus which it would seem must follow the removal of one substance. On the other hand, vacuolation frequently occurs in some nucleoli (Fikry (9) on *Rumex*) and this might be due to the removal of some substance from the nucleolus. The possibility of indirect contribution is not therefore removed, but the evidence in support of the ergastic nature of the nucleolus must also be considered.

Among investigators who consider that the nucleolus is an ergastic substance are Lundegårdh (30) and Meyer (33). Both bring forward very good evidence in favour of their view; Lundegårdh by his work on living material, and Meyer by finding the effect of nutrition. Meyer made the interesting discovery that lack of food substances caused a reduction in size of the nucleolus. He also found that the cells appeared to divide normally, so that the nucleolus appears to be unessential in mitosis.

Judging by staining reactions, chromatin seems to be absent from the nuclear sap, so that it must originate by the building up or breaking down of some other substance. Now, this would involve chemical action, and some agent capable of bringing about such action must be sought. Fikry (9) has suggested that the chromatin is produced by the action of a number of enzymes, but the production of these enzymes during cell division must be accounted for, and their presence has yet to be demonstrated.

Water is present throughout the plant, and may be concerned in bringing about the appearance of the chromatin. It does not seem impossible that the ionization of some electrolyte may be responsible for the deposition of chromatin on the linin thread, for several workers (Farmer and Digby (8)) have found that the linin thread is electrically charged, while Zirkle (39) and others maintain that the charge is negative. Any positively charged ions present in the solution would then tend to be deposited on this, and, since chromatin is gradually deposited on the threads, it seems that it may be composed of cations resulting from the ionization of some more complex substance. The fact that the chromatin is definitely deposited on the linin threads and not on any particles in the sap suggests that the deposition is not due to simple precipitation or saturation, but to some definite relation between the chromatin and the linin threads. The anions produced by the ionization would, on the other hand, tend to move away from the thread, and would travel towards the periphery, or perhaps towards the nucleolus, which appears to be positively charged (Zirkle (39)).

If the nucleolus were made up of two substances, or of one easily dissociated substance, it is possible that, as the chromatin is deposited and the solution becomes devoid of cations, some part of the nucleolus would tend to pass into solution to counteract this deficiency. This would account for the fact that the nucleolus gradually changes its staining reactions as the threads become thickened. In this case it might not be of importance whether or not the substance were chromatin, for it would merely take the place of the cations in the sap. Whatever the composition of the nucleolus, it alone cannot be responsible for the thickening of the threads, since this begins before there is any apparent change in the nucleolus, and the amount of chromatin added may be greater than the volume of the nucleolus.

Thickening begins while the thread is still in contact with the membrane, and if, as Koerperich (21) maintains, there is definite contact between the chromosomes and the cytoplasm, then it seems likely that the anions which are liberated in the vicinity of the thread will tend to stream out into the cytoplasm through these points of contact. As the linin is negatively charged it will tend to repel the rest of the thread, but as this charge becomes neutralized by the deposition of chromatin the repulsion will be lessened, and the threads will tend to take up a central position, away from the anions. The streaming of these ions having once begun, would tend to go on in the same course, so that they would continue to pass out in the direction of the points of contact, even when the threads have become detached from the membrane.

*Orientation of the chromosomes and the spindle.*

It has often been maintained that the spindle fibres grow inwards towards the chromosomes and are responsible for their orientation. In *Oenothera*, however, the orientation of the chromosomes takes place before the disappearance of the nuclear membrane and the appearance of the spindle (Pl. II, Figs. 12, 19, 20) so that in this case such an explanation will not hold, and it is evident that the orientation must be internal to the nucleus. It is perhaps significant that in cases where the spindle is multi-polar the fibres should appear just opposite the groups of chromosomes, even before the membrane has disappeared (see Farmer and Digby (8), Figs. 17, 27, &c.). Such a state could be readily explained on the assumption that the anions were streaming out through the points of contact of the chromosomes, and that their appearance as fibres was due to the fixative causing their precipitation (cf. Martens (32)).

Resuming briefly: It is suggested that the chromosomes become thickened by deposition of some substance which arises, in part at least, from a substance present in the nuclear sap, the nucleolus passing into solution. The deposition may be due to the ionization of a substance, the cations becoming deposited on the negatively charged linin, and the anions streaming outward and possibly by their precipitation giving rise to the appearance of spindle fibres, which represent the forces, originating in the nucleus, which are responsible for the orientation of the chromosomes within the nucleus.

## PART II.

*Catenation.*

In the section on meiosis a general description applicable to all the forms studied was given. At diakinesis, however, the various hybrids show important differences, so that each must now be treated in turn.

In the various  $F_1$  cultures it was found that the plants could usually be grouped into two types, this being particularly easy in the hybrid *O. rubricalyx*  $\times$  *O. blandina*. One type was pale green in colour, while the other was considerably darker, and Gates and Catcheside (13) suggest that this is due to the presence of two different complexes in *O. rubricalyx*, one carrying a factor for light green, *rubricalyx*  $\alpha$ , and one carrying a factor for dark green, *rubricalyx*  $\beta$ . Where it was possible to identify the two types the catenation was determined in representatives of each type, and the results compared. In the case of *O. nutans*, too, Gates and Catcheside (13) have recognized two complexes, *nutans* and *serratans*. Table I gives a summary of the results obtained, and where possible the complex has been mentioned. The catenation in the parents as well as that of the hybrids is given in each case.

TABLE I.

*Catenation in Some Crosses of O. rubricalyx.*

Hybrid.	Catenation.	Catenation in female parent.	Catenation in male parent.
<i>Rubricalyx</i> $\alpha$ <sup>h</sup> <i>deserens</i>	Ring of 4, 5 pairs	Ring of 6, 4 pairs	Ring of 7 pairs
<i>Rubricalyx</i> $\beta$ <sup>h</sup> <i>deserens</i>	" 4, 5 "	" 6, 4 "	" 7 "
<sup>h</sup> <i>Deserens. rubricalyx</i> $\alpha$	" 4, 5 "	" 7 "	" 6, 4 "
<sup>h</sup> <i>Deserens. rubricalyx</i> $\beta$	" 4, 5 "	" 7 "	" 6, 4 "
<i>Rubricalyx</i> $\alpha$ <sup>h</sup> <i>purpurata</i>	" 6, 4 "	" 6, 4 "	" 7 "
<i>Rubricalyx</i> $\beta$ <sup>h</sup> <i>purpurata</i>	" 6, 4 "	" 6, 4 "	" 7 "
<sup>h</sup> <i>Purpurata. rubricalyx</i> $\beta$	" 6, 4 "	" 7 "	" 6, 4 "
<i>Rubricalyx</i> $\beta$ . <i>nutens</i>	" 8, 3 "	" 6, 4 "	" 14
<i>Serratans. rubricalyx</i> $\alpha$ (Catcheside, 1933)	" 12, 1 "	" 14	" 6, 4 pairs
<i>O. rubricalyx. ammophila</i> (F <sub>2</sub> )	" 6, 4 "	" 6, 4 pairs	" 12, 1 "
<i>Rubricalyx</i> $\alpha$ <sup>h</sup> <i>blandina</i>	" 4, 5 "	" 6, 4 "	" 7 "
<i>Rubricalyx</i> $\beta$ <sup>h</sup> <i>blandina</i>	" 8, 3 "	" 6, 4 "	" 7 "
<i>O. blandina. rubricalyx</i>	" 4, 5 "	" 7 "	" 6, 4 "
Trisomic Mutant (from <i>rubricalyx</i> $\beta$ <sup>h</sup> <i>blandina</i> )	" 8 + 1, 3 "	" 6, 4 "	" 7 "

*O. rubricalyx*  $\times$  *O. deserens*. (Pl. II, Figs. 8, 9).

The plants in this culture were in general very similar, but owing to slight difference they were classed into two types (Gates and Catcheside, (13)). One plant from each type was examined and both showed the same configuration—a ring of four chromosomes and five pairs: in nearly every case all the pairs being ring pairs. There were many cases in which pairs were interlocked with each other and with the ring, and some attempt was made to form an estimate of the various kinds of interlocking. It was found that the interlocking was both of the distal type—in which the connected ends of the chromosomes are linked with each other, and of the medial type—in which the chromosomes are linked near the centre.

With a ring of four chromosomes and five pairs it is possible to have a great variety of interlocking, and a short table (Table II) is given to show the great variety of interlocking observed in this form. From the results given it appears that all kinds of interlocking may occur, and that one type is not noticeably more frequent than any other, so that it seems that interlocking is accidental, and that it is of no great importance (cf. Catcheside, (1)).

*O. deserens*  $\times$  *O. rubricalyx* (Pl. II, Fig. 10).

In this culture it was possible to make out the same two types as in the reciprocal. Material was collected from four plants of the  $\beta$  type and from one plant of the  $\alpha$  type. In all five plants examined the catenation was the same as in the reciprocal, a ring of four chromosomes and five pairs.

TABLE II.

*Interlocking of Chromosomes in O. rubricalyx* × *O. deserens*.

(Each interlock has been counted so that where one pair is interlocked in two places both interlocks are considered.)

No. of pairs interlocked with ring.	No. of pairs interlocked together.	No. of free pairs.	No. of observations.
5	0	0	2
4	0	1	1
3	2 + 2	0	1
3	3	1	3
3	2	1	2
3	0	2	1
2	3	2	1
2	3	0	1
2	2	2	1
2	2	1	2
2	0	3	1
1	3 + 2	0	1
1	2 + 2	1	1
1	2 + 2	0	1
1	3	2	1
1	3	1	3
1	2	3	1
1	2	2	2
1	0	4	2
0	4	1	1
0	3	2	1
0	2	3	2

*O. rubricalyx* × *O. purpurata* (Pl. II, Figs. 11, 12).

The plants in this culture showed great uniformity, but it was possible to see a slight difference in leaf colour, indicating the influence of the two complexes. Material from each type was collected, and the catenation in both was found to be a ring of six chromosomes and four ring pairs, with sometimes a chain instead of a ring. Pl. II, Fig. 11, is from a plant of the  $\beta$  type, and Plate II, Fig. 12, from a plant of the  $\alpha$  type.

*O. purpurata* × *O. rubricalyx*. (Pl. II, Fig. 13).

This culture was quite uniform and resembled the reciprocal in all respects. Material was collected from one plant ( $\beta$  type) but no case of diakinesis was found. In metaphase and early anaphase it was possible to find four pairs of chromosomes in many cases, and in several mother-cells it could be seen that the catenation was a ring of six chromosomes and four pairs, as in the reciprocal.

*O. rubricalyx* × *O. nutans* (Pl. II, Figs. 14, 16).

Unfortunately the plants of this culture did not develop. Material was collected from two plants which had been identified as this hybrid. The buds were fixed in La Cour's 2B, but this was not suitable as the material was much shrunk, making it very difficult to determine the catenation. In only one case was a complete ring of eight chromosomes

observed, but numerous cases were seen in which there was a chain of eight chromosomes and three ring pairs. It seems very probable that the violent action of the fixative was responsible for breaking the large ring, and this may be the explanation of irregularities observed. The complex combination in this hybrid was *rubricalyx*  $\beta$ . *nutans* (Gates and Catcheside, (13)).

*O. nutans*  $\times$  *O. rubricalyx*.

The plants in this culture differed from the reciprocal, the complexes involved being *serratus* and *rubricalyx*  $\alpha$ , and the catenation a ring of twelve chromosomes and one pair (Catcheside (2)).

*O. rubricalyx*  $\times$  *O. ammophila*  $F_2$  (Pl. II, Fig. 15).

This culture, which was the  $F_2$  generation, consisted of plants in which it was impossible to trace definitely the influence of the complexes. The catenation was a ring of six chromosomes and four pairs.

*O. rubricalyx*  $\times$  *O. blandina* (Pl. II, Figs. 17, 18).

This proved to be the most interesting culture, for it was possible to show distinctly the influence of the two complexes in *O. rubricalyx*. On examination of the paler type it was found that the configuration at diakinesis was a ring of four chromosomes and five pairs (Pl. II, Fig. 17). In the darker type the configuration was a ring of eight chromosomes and three pairs (Pl. II, Fig. 18). Since *O. blandina* is homozygous, this difference in configuration must be due to the presence of the two different complexes in *O. rubricalyx*, and this confirms the idea that *O. rubricalyx* is heterozygous. In this culture, therefore, it is possible to trace the effect of the two complexes both cytologically and genetically.

*O. blandina*  $\times$  *O. rubricalyx* (Pl. II, Figs. 19, 20).

It was difficult to recognize two distinct types in this culture and material was collected from three plants apparently of the same type. Only two of these showed diakinesis, and in both cases the catenation was a ring of four chromosomes and five pairs, as in the  $\alpha$  type of the reciprocal.

In all the cultures examined interlocking was frequent, and it varied greatly, as already prescribed for *O. rubricalyx*  $\times$  *O. deserens*.

*Trisomic mutant.*

In the culture of *O. rubricalyx*  $\times$  *O. blandina* which numbered eighty plants, were two mutants. One was examined by Mr. D. G. Catcheside, and for the other he kindly furnished the following details. This plant differed from the type principally in having broader, blunter, deeper green leaves which were characteristically oblong, but Professor Gates determined

that it was not *oblonga*. The bracts were broadly elliptical, quite smooth, and had a practically entire margin. The stem was red and rather weak. The buds and hypanthia were paler and shorter than in the corresponding diploid type, and they dropped off before flowering, so that actual flowers were never seen. On examining the pollen Mr. Catcheside found only 28 per cent. of good pollen, and a large number of four-lobed grains.

A considerable amount of material was collected and all the buds were examined, with the following results:

TABLE III.

Stage.	No. of Buds.
Prophase	7
Diakinesis	2
Anaphase	2
Tetrads	6
Grains	1
Sterile	7
Total	<hr/> 25

The buds showing prophase did not differ greatly from those of other plants examined, but sometimes one or two loculi were seen to contain pollen mother-cells having very large nuclei and extremely thin walls. In the sterile buds examined these features were also seen in some of the loculi, while in others there were large cells devoid of cytoplasm and containing only a small collapsed nucleus in one corner. It seems that the cessation of development during prophase results in the production of totally sterile buds, for in no buds with sterile loculi was any stage later than prophase observed. As fertile and sterile buds are present together, it seems that the factor arresting normal development must be internal to the bud.

In normal developing pollen mother-cells there is at first an increase in the volume of the cytoplasm followed by a considerable increase in volume of the nucleus. In this mutant, however, the nucleus apparently grew first at the expense of the cytoplasm. The cause of the sterility seems to be lack of nutrition, and since other buds in the inflorescence had developed normally, the failure must be in the bud itself, and a possible cause is a defective vascular system.

Several of the somatic cells showed metaphase plates, and chromosome counts showed that fifteen chromosomes were present, and that the mutant was therefore trisomic (Pl. II, Fig. 21). In several chromosomes a medial constriction could be seen, and it was found that the plate consisted of nine rather bent chromosomes and six nearly straight ones. Two other plates examined showed the same distinction between the chromosomes.

During diakinesis the tapetum was found to be binucleate, as in

other forms, and many cases were seen in which three or more nuclei were present. In several metaphase plates there were a very large number of chromosomes. In one fifty-six were counted, so that this nucleus was octoploid.

The buds showing diakinesis were studied in detail. Undoubtedly the most usual configuration was a chain of nine chromosomes and three pairs (see Table IV). It is interesting to compare this result with the somatic metaphase plates, for it was found there that the chromosomes fell into two groups, one consisting of nine bent chromosomes, and the other of six straight chromosomes. Possibly the nine bent chromosomes are those which form the chain, while the six straight ones form the three pairs.

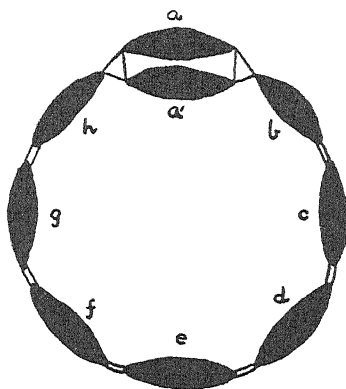
Besides a chain of nine chromosomes, it was found that there was sometimes a chain in which two chromosomes were connected to the same chromosome. In other cases a chain of seven chromosomes and four pairs were found, and many other variations with shorter chains were found, but these were often due to injury to the nucleus by cutting. In two cases there was a closed ring of eight chromosomes and three pairs. In these cases the ninth chromosome was seen to be attached to the ring (Pl. II, Fig. 23). It seems then that the ninth chromosome may be attached to one of the chromosomes in the ring and also to the two neighbouring chromosomes (e.g. Pl. II, Fig. 27). There must, therefore, be two connexions at the end of this chromosome, and it seems that each chromosome may have two connexions, a fact which it is very difficult to demonstrate in the disomic forms.

Since cases have been observed in which there is a ring of eight chromosomes to which the ninth is attached, it seems that this must be the correct configuration in this mutant, for all the other configurations can readily be derived from it. In all cases there are three pairs, so that these need not be taken into consideration in this connexion.

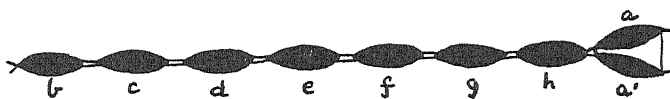
Text-fig. 1 is an illustration of the ideal arrangement of the chromosomes. Small single letters have been used to denote the chromosomes as it is not intended to make any reference to definite chromosomes. In the diagram double connexions have been indicated between all the chromosomes as there is some evidence of their occurrence here and in other forms (Catchside (1)). All the chromosomes, except  $a$  and  $a'$ , and one end of  $b$  and  $b'$ , are then connected to each other by parallel double connexions. Earlier in this paper it has been suggested that the chromosomes are drawn towards the membrane (and away from each other) by forces acting chiefly at their central point. If this is the case, it seems likely that the connexions between  $a$ ,  $a'$ ,  $b$  and  $b'$  would break more readily than the others, since here there is only one connexion in any one direction. If, for instance, the connexion between  $a$  and  $b$  were broken it seems likely that the next break would be between  $a$  and  $a'$  or  $a'$  and  $b$ . It would be nearly



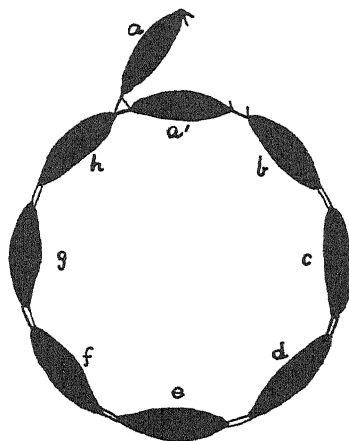
impossible to show the absence of one connexion, as this would make no apparent difference in the configuration. Two breaks, however, should be more readily seen. If the second break occurred between  $a'$  and  $b$ , then a



TEXT-FIG. 1.



TEXT-FIG. 2.



TEXT-FIG. 3.

chain of seven chromosomes having a terminal pair attached would result (Text-fig. 2). This has been observed in three cases (Pl. II, Fig. 24).

If, on the other hand, the second break occurred between  $a$  and  $a'$  then a ring of eight chromosomes with a ninth attached at one end only would result (Text-fig. 3). This has also been observed, and is illustrated by Pl. II, Fig. 23.

It is, of course, immaterial at which end of  $a$  and  $a'$  the breaks have

taken place as the resulting configuration will be the same. In Text-fig. 2 only one connexion remains between  $a$  and  $a'$ , and in Text-fig. 3 only one connexion remains between  $a'$  and  $b$ , so that it seems that these would readily break, in both cases resulting in a chain with a forked pair at the end. Two such cases were observed, and one is figured (Pl. II, Fig. 25).

It is possible that one of the double connexions between the chromosomes will break, and since the chromosomes appear to be equivalent any of the connexions might break. Such breaks would, from Text-fig. 2, result in short chains having a pair at the end of one of the chains. Several of these were seen. This condition could also have arisen by the breaking of the connexion between  $a'$  and  $b$  in Text-fig. 3, followed by a break elsewhere, so that no importance can really be attached to the frequency with which this is observed. On the other hand, the only possible way in which a chain with a centrally placed pair could arise is by the breaking of a connexion in Text-figs. 1 or 3, and the chances of distribution of the chromosomes each side of the pair should therefore be equal, so that there would be equal numbers of chains showing the following arrangements:

1 chromosome, pair, 6 chromosomes (Pl. II, Fig. 27) 2 cases seen.

2       "       "       5       "       3       "       "  
3       "       "       4       "       (P. II, Fig. 28) 2       "       "

The most frequent arrangement was a chain of nine chromosomes, and by looking at the Text-figs. it is possible to see that it might be derived from either arrangement. From Text-fig. 2 by the breaking of the connexion between  $a$  and  $a'$  near  $h$ , and either between  $a$  and  $h$  or  $a'$  and  $h$ , while in the case of Text-fig. 3 it may result from the breaking of the connexions between  $a$  and  $a'$ , and  $a'$  and  $h$ , or between  $a$  and  $h$ , and  $a'$  and  $h$ .

The accompanying table gives the configurations involving not more than two chains. In each case three pairs are present in addition.

TABLE IV.

Arrangement.	No. seen.	Illustration.
Chain of 9 chromosomes . . . . .	24	Fig. 22
Ring of 8 with 1 chromosome attached . . . . .	2	Fig. 23
Chain of 8 with 1 chromosome attached . . . . .	7	Figs. 27, 28
Chain of 7 with 1 ring pair attached . . . . .	2	Fig. 24
Chain of 7 with 1 open pair attached . . . . .	2	Fig. 25
Chain of 7 with 1 free ring pair . . . . .	8	Fig. 26
Chain of 7, chain of 2 chromosomes . . . . .	2	
Chain of 6, 1 pair attached, 1 free chromosome . . . . .	4	
Chain of 6, chain of 3 chromosomes . . . . .	2	
Chain of 5, chain of 2, 1 pair attached . . . . .	1	
Chain of 5, chain of 4 chromosomes . . . . .	4	

Smaller chains than those given in the table were observed, but these were present in cut nuclei.

Regarding the origin of the fifteenth chromosome, the fact that it is connected at both ends to one of the chromosomes in the ring indicates that it is homologous with this chromosome. Non-disjunction is a fairly common phenomenon in *Oenothera*, and probably accounts for the origin of this trisomic form (Gates (11)). Another possibility is the duplication of one of the chromosomes already in the complex, by division. In *O. deserens*  $\times$  *O. rubricalyx* pollen mother-cells were seen in early telophase to contain eight chromosomes in one daughter nucleus and seven in the other (Pl. II, Fig. 7). But two of the chromosomes were single while the others were double, so that one chromosome must have divided earlier than the others. The two halves seemed, however, to be larger than the other half chromosomes and possibly each would divide, resulting in two pollen grains having eight nuclei. In a homozygous form this would ultimately give the same results as non-disjunction, but in heterozygous forms the duplicated chromosome would be one already present in the complex, whereas in non-disjunction the extra chromosome might be one belonging only to the other complex. It was not possible to obtain any conclusive evidence as to the fate of this chromosome, but there were indications, from metaphase, that the two chromosomes might both pass to the same pole.

Although the seven-eight distribution is that most commonly found in trisomic forms, Gates and Thomas (16) have shown that six-nine distribution may also occur, and this was again observed in the present mutant. One case was seen in which the daughter nuclei contained nine and eight chromosomes respectively (Pl. II, Fig. 29). Here, again, it seems that premature separation of the halves is responsible, but it may be that one of the chromosomes divided before passing to the poles, for each daughter nucleus contains one chromosome which seems smaller than the others, and these may represent the two halves of the same chromosome, so that one nucleus may contain a chromosome usually absent from that complex, just as in non-disjunction.

One pollen mother-cell was seen to have thirty chromosomes at metaphase (Pl. II, Fig. 30). The chromosomes are definitely arranged in ring pairs so that there are fifteen pairs of homologous chromosomes. Presumably this tetraploid cell is the result of failure of the chromosomes to separate after dividing at the last premeiotic division. Similarly evidence of non-disjunction in the last premeiotic mitosis or earlier has been obtained by Hedayetullah (19) in *O. rubricalyx*  $\times$  *O. eriensis*.

Besides differing in chromosome number and arrangement, this cell differs greatly in size from the neighbouring pollen mother-cells which are of normal size and show the usual catenation. Measurements of several cells at the same stage were taken, and it was found that the area of the optical medial section of this cell was just twice the area of a normal

pollen mother-cell, while the volume (reckoned as a sphere) was nearly three times that of a normal cell.

The trisomic mutant, then, although showing a variety of configurations, appears to behave quite normally at diakinesis, for it is possible to interpret all the configurations as derived from a branched ring of eight chromosomes and three pairs of chromosomes.

#### *Configuration at diakinesis.*

From the results of numerous workers on *Oenothera* there appears to be good reason for suggesting that the configuration is generally constant in any one plant and in all plants of that type. There is, however, evidence that this is not universally true. Miss Sheffield (36) found several irregularities, and lately Miss Leliveld (26) maintains that the catenation is not constant, and she gives figures showing the variety of configuration obtained. From the present observations it seems that many apparent irregularities can be accounted for by the action of fixatives, the cutting of the nucleus, or even the interpretation of the observations. The hybrid *O. rubricalyx*  $\times$  *O. eriensis* has been examined in great detail (Hedayetullah (19)) and here the configuration varies widely, so that it appears that there is, in certain forms at least, a considerable amount of variation. Among the forms examined, the only cases of undoubted variation occurred in the mutant, and here some explanation has been given as to its origin. It is possible that irregularity of configuration may result in non-disjunction.

#### THEORETICAL CONSIDERATIONS.

The most favoured theory of the chromosome configuration is at present that of segmental interchange, which has been expounded and discussed at length by Darlington (6) and Cleland and Blakeslee (5). The chromosome constitution of *rubricalyx* has already been discussed on this basis (Gates and Catcheside (13), p. 171) and need not be further considered here.

Using the nomenclature adopted by Gates and Catcheside (1932), from available crosses, they concluded that the possible formulae for *rubricalyx*  $\alpha$  were :

$$\begin{array}{l} (1) \text{ A.B} \quad \text{D.G} \quad \text{C.H} \\ (2) \text{ C.D} \quad \text{A.H} \quad \text{B.G} \end{array} \left. \vphantom{\begin{array}{l} (1) \\ (2) \end{array}} \right\} \text{E.F} \quad \text{K.L} \quad \text{M.N} \quad \text{O.P}$$

and for *rubricalyx*  $\beta$

$$\begin{array}{l} (1) \text{ D.E} \quad \text{F.G} \quad \text{A.H} \\ (2) \text{ D.F} \quad \text{E.G} \quad \text{A.H} \\ (3) \text{ D.G} \quad \text{A.F} \quad \text{E.H} \\ (4) \text{ D.G} \quad \text{A.E} \quad \text{F.H} \end{array} \left. \vphantom{\begin{array}{l} (1) \\ (2) \\ (3) \\ (4) \end{array}} \right\} \text{K.L} \quad \text{M.N} \quad \text{O.P} \quad \text{B.C.}$$

Formula (1) for the *rubricalyx*  $\alpha$  complex can only go with formulae (3) or (4) for *rubricalyx*  $\beta$  to give *O. rubricalyx* with a ring of six chromosomes. Similarly, formula (2) for  $\alpha$  will only go with (1) or (2) for  $\beta$ .

Since the trisomic mutant has a configuration consisting of nine chromosomes, the extra being attached to the ring of eight (Text-fig. 1) this chromosome must be a duplicate of one in the ring. The trisomic probably arose through non-disjunction in the ring of six chromosomes in the megaspore of *rubricalyx*  $\alpha$ , since: (1) non-disjunction of bivalent chromosomes is rare, and (2) the evidence also indicates that eight-chromosome pollen grains are much less likely to function because of the competition with the pollen tubes of grains having seven-chromosome nuclei.

Some attempt can now be made to identify this extra chromosome. Using the above nomenclature, and the formula for <sup>h</sup>*blandina* as A.B C.D E.F G.H K.L M.N O.P (Gates and Catcheside, (13)), chromosomes K.L M.N O.P are excluded, since they do not take part in the ring in the trisomic. The  $\alpha$  complex plus any extra chromosome from the  $\beta$  complex is excluded as the eight-chromosome female gamete, since it cannot give the correct configuration with <sup>h</sup>*blandina*. The eight-chromosome female gamete must therefore have contained the  $\beta$  complex plus a ring-forming chromosome of the  $\alpha$  complex. This extra chromosome must be similar to one of the *blandina* chromosomes taking part in the ring. Now <sup>h</sup>*blandina* and *rubricalyx*  $\alpha$  have E.F in common, and either A.B or C.D; hence one of these three chromosomes is probably the extra chromosome present in the trisomic.

#### SUMMARY.

1. The catenation is studied in a series of F<sub>1</sub> hybrids of *O. rubricalyx* (see Table I).
2. An examination of the prophase stages leads to the conclusion that the resting nucleus consists of a mass of threads connected by anastomoses which gradually break during development.
3. The darkly staining bodies in the reticulum appear to be of two kinds, those due to crossing of the threads, and those representing the points of attachment of the chromosomes.
4. It is suggested that although synizesis is usually an artifact it may be produced in nature by some alteration in conditions; but it is neither a universal nor an important stage in meiosis.
5. Evidence is given in support of the view that several spiremes are present in heterotypic prophase, corresponding in number to the rings apparent at diakinesis.
6. The physico-chemical relations of the nuclear sap, the chromatin, and the nucleolus are discussed with reference to chromosome growth.

7. Variations in the catenation of a trisomic mutant from *O. rubricalyx* × *O. blandina* are explained as derived from one configuration in which one chromosome appears to be duplicated.

8. A single very large pollen mother-cell of this mutant was found having thirty chromosomes in ring pairs. It probably originated from chromosome doubling in the last premeiotic mitosis.

9. The relative constancy of the various configurations makes it possible for the catenation to be explained on the hypothesis of segmental interchange, and this is used as a basis for determining the segmental arrangement of the two complexes present in *O. rubricalyx*.

The work was carried out in the Botanical Department of King's College (University of London), under the supervision of Professor Gates, to whom I wish to tender my very grateful thanks for his valuable help and advice, and for having arranged this paper in a form suitable for publication.

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## EXPLANATION OF PLATE II.

Illustrating Miss M. Verbrugge's paper on 'Meiosis and Catenation in Certain Crosses of *Oenothera rubricalyx*'.

All figures were drawn with the aid of a camera lucida at table level, with 2 mm. immersion Zeiss N. A. 1.4. A Zeiss ocular No. 18 was used. The magnification is  $\times 3,300$ .

The following convention has been adopted:—All chromosomes in top focus have been drawn in black, those in middle focus have been stippled, and those in lower focus have been left in white.

Fig. 1. *O. deserens* × *O. rubricalyx*. Nucleus in early prophase showing reticulum with thickenings and vacuolate nucleolus.

Fig. 2. *O. deserens* × *O. rubricalyx*. Nucleus showing beaded thread.

Fig. 3. *Rubricalyx* α. <sup>h</sup>*blandina*. Late thread stage. I, II forming small ring presumably of two chromosomes, and III, IV, V representing three chromosomes in a larger ring or chain. (The thread has been drawn slightly thicker than it appears for clearness.)

Fig. 4. *Rubricalyx* α. <sup>h</sup>*blandina*. Nucleus showing endonucleolus divided into two.

Fig. 5. *O. deserens* × *O. rubricalyx*. Five small rings, I, II, III, IV, V, probably representing five ring pairs, and one larger ring VI, probably representing a ring of four chromosomes, can be made out.

Fig. 6. *O. deserens* × *O. rubricalyx*. Nucleus showing five ring pairs and one ring of four chromosomes. Several darkly staining masses are present in the cytoplasm.

Fig. 7. *O. deserens* × *O. rubricalyx*. Heterotypic telophase. Upper nucleus showing seven split chromosomes, lower one showing six split chromosomes and two half chromosomes.

Fig. 8. *O. rubricalyx* × *O. deserens*. Diakinesis, showing a ring of four chromosomes, three free pairs and two interlocked pairs.

Fig. 9. *O. rubricalyx* × *O. deserens*. Diakinesis. All five pairs are interlocked with the ring.

Fig. 10. *O. deserens* × *O. rubricalyx*. Diakinesis, showing ring of four chromosomes with three interlocked pairs, and two free pairs. Two darkly staining nucleoli are present.

Fig. 11. *O. rubricalyx* × *O. purpurata*. Diakinesis, with one pair interlocked with the ring of six chromosomes, and three free pairs.

Fig. 12. *O. rubricalyx* × *O. purpurata*. Nucleus showing the beginning of orientation. The membrane and nucleolus are still present. One of the chromosomes appears as if it were being pulled outwards at its central point.

Fig. 13. *O. purpurata* × *O. rubricalyx*. A ring of six chromosomes and four pairs are seen on the spindle.

Fig. 14. *O. rubricalyx* × *O. nutans*. A chain of eight chromosomes and three pairs are seen centrally placed, but neither membrane nor spindle fibres are visible.

Fig. 15. *O. rubricalyx* × *O. ammophila*, F<sub>2</sub>. Catenation, showing a ring of six chromosomes and four pairs, in one of which the ring has apparently broken.

Fig. 16. *O. rubricalyx* × *O. nutans*. Chromosomes have begun to move towards the poles, for several of the connexions are broken. It is still possible to make out the three ring pairs.

Fig. 17. *Rubricalyx* α. <sup>h</sup>*blandina*. Diakinesis, showing a ring of four chromosomes and five pairs.

Fig. 18. *Rubricalyx* β. <sup>h</sup>*blandina*. Diakinesis, showing a ring of eight chromosomes and three pairs.

Fig. 19. *O. blandina* × *O. rubricalyx*. The ring of four chromosomes and the five pairs are beginning to show orientation.

Fig. 20. *O. blandina* × *O. rubricalyx*. A later stage in the orientation. Both the membrane and nucleolus are present.

#### Trisomic Mutant

(from *Rubricalyx* β. <sup>h</sup>*blandina*)

Fig. 21. Somatic plate showing nine bent chromosomes and six nearly straight chromosomes. Constrictions are visible in nearly all.

Fig. 22. Diakinesis, showing a chain of nine chromosomes with which are interlocked the three pairs.

Fig. 23. A ring of eight chromosomes is somewhat twisted, and a ninth chromosome is attached by one of its ends to the ring. Two pairs are interlocked with the ring and the third pair may be distally interlocked with it.

Fig. 24. Four ring pairs are present, one of which appears to be attached to the end of the chain of seven chromosomes.

Fig. 25. An open pair is attached to the end of the chain of seven chromosomes and three free pairs are present.

Fig. 26. Nucleus with four ring pairs and a chain of seven chromosomes. The nucleolus is also present.



Fig. 27. One chromosome is attached to the end of a ring pair while a chain of six chromosomes is attached to the other end. Three ring pairs are also present.

Fig. 28. The nucleus is cut and only a small portion of each of two pairs can be seen. Another pair is uncut and is interlocked with the chain. This consists of a chain of three chromosomes attached to the end of a pair, and a chain of four chromosomes attached to the other end. The nucleolus is very granular.

Fig. 29. The daughter nucleus on the right appears to contain nine chromosomes while that on the left has eight. In each, one chromosome seems slightly smaller than the others, so that it may be the result of division before separation.

Fig. 30. Metaphase spindle from a tetraploid pollen mother-cell showing fifteen ring pairs of chromosomes.







# Some Observations on the Genus *Cladochytrium* with Special Reference to *C. caespitis* Griffon and Maublanc.

BY

W. R. IVIMEY COOK, PH.D.

With Plate III and nine Figures in the Text.

IN December, 1932, I received from Dr. G. H. Pethybridge a sod of fairly recently sown turf containing seedlings of a species of *Agrostis*, a large number of which were attacked by a parasitic fungus that caused their browning and ultimate death. This material had been sent to the Plant Pathological Laboratory of the Ministry of Agriculture from Yorkshire. Examination of the affected seedlings showed that they were attacked by a fungus whose characteristic symptom was the development of large resting sporangia in the cells of the roots and sheath and, to a less extent, in the cells of the leaves as well.

Dr. Pethybridge informed me that following Massee's (6) record of *Cladochytrium graminis* Büsgen as a new grass parasite in Britain in 1913, it had been customary in this country to regard this species as accountable for the diseased areas not infrequently arising in recently sown lawns, tennis grounds, and bowling greens, when an obviously Chytridiaceous parasite occurs in the roots of the young grasses. He thought, however, that the appearance of the organism in the present instance, at any rate, did not quite agree with that described by Massee, but resembled very strongly the *C. (Physoderma) caespitis* described by Griffon and Maublanc (2) in 1910. He therefore asked me to look into the matter.

With the assistance of Dr. Pethybridge and through the kindness of the Director, the several sheets of *C. graminis* Büsgen in the Kew herbarium were placed at my disposal for examination. Six packets of specimens were preserved on the sheets, obtained chiefly from the continent of Europe; but one from Surrey was present, which appeared to have been the material on which Massee based his record and account of the organism. No information was appended as to the species of grass attacked. Examination of this material showed that the fungus occurred chiefly in the roots, but in all the other collections the parasites

described are found occurring in the leaves. The following grasses were represented: *Dactylis glomerata*, *Alopecurus pratensis*, and *Triticum repens*. Massee (6) states that, while the seeds of *Poa annua* and *Festuca ovina* sown in infected soil become attacked, those of *D. glomerata* and *T. caninum* remained free.

A preliminary microscopic examination of the material suggested that the specimens were not all of the same fungus in so far as the resting sporangia were concerned, and their size was accordingly measured, with the following results:

Kew ref.	Host plant.	Average sporangium size.	Number measured.
71	<i>Dactylis glomerata</i>	$31.2 \times 29.5 \mu$	24
72	<i>Triticum repens</i>	$30.2 \times 26.8 \mu$	25
4177	<i>Dactylis glomerata</i>	$36.2 \times 33.2 \mu$	26
4926	<i>Dactylis glomerata</i>	$35.8 \times 30.4 \mu$	26
1827	<i>Alopecurus pratensis</i>	$36.4 \times 30.7 \mu$	26
(Surrey)	?	$34.2 \times 25.8 \mu$	10

From these measurements it will be seen that the material from Surrey possessed, on an average, more elongated resting sporangia than those of the other examples. Moreover, these sporangia were found in the roots, whereas all the others occurred in the leaves. The Yorkshire material now under consideration had resting sporangia whose average size was  $30.7 \times 14.8 \mu$  (average of  $30 \mu$ ), that is to say, they were even more elongated than those from the Surrey material.

In 1910 Griffon and Maublanc (2) described a species of *Cladochytrium*, which they called *C. (Physoderma) caespitis*, attacking the sheath and adjacent tissues of *Lolium perenne*. In this species the resting sporangia varied from  $15$  to  $45 \times 12$  to  $30 \mu$ .

In addition to the difference in the size of the sporangia these authors state that their organism differs from *C. graminis* Büsgen, with which they compared it, in the fact that the spore walls are almost colourless. This latter character is also shared by the Kew material from Surrey and in the present examples, but is not found in any of the other examples.

In 1901 Lagerheim collected material of another species which is referred to by von Minden (7) as *Physoderma Agrostidis* Lag. This species was found in the leaves of *Agrostis alba*. The sporangia are described as being more or less ellipsoidal,  $13-21 \times 17-25 \mu$  in size, with yellow-brown membranes. Through the kindness of Mr. Ramsbottom the writer was able to obtain type material of this species from the herbarium of the British Museum. In the material so obtained the resting sporangia measured  $18.5 \times 24.4 \mu$  (average of  $26 \mu$ ).

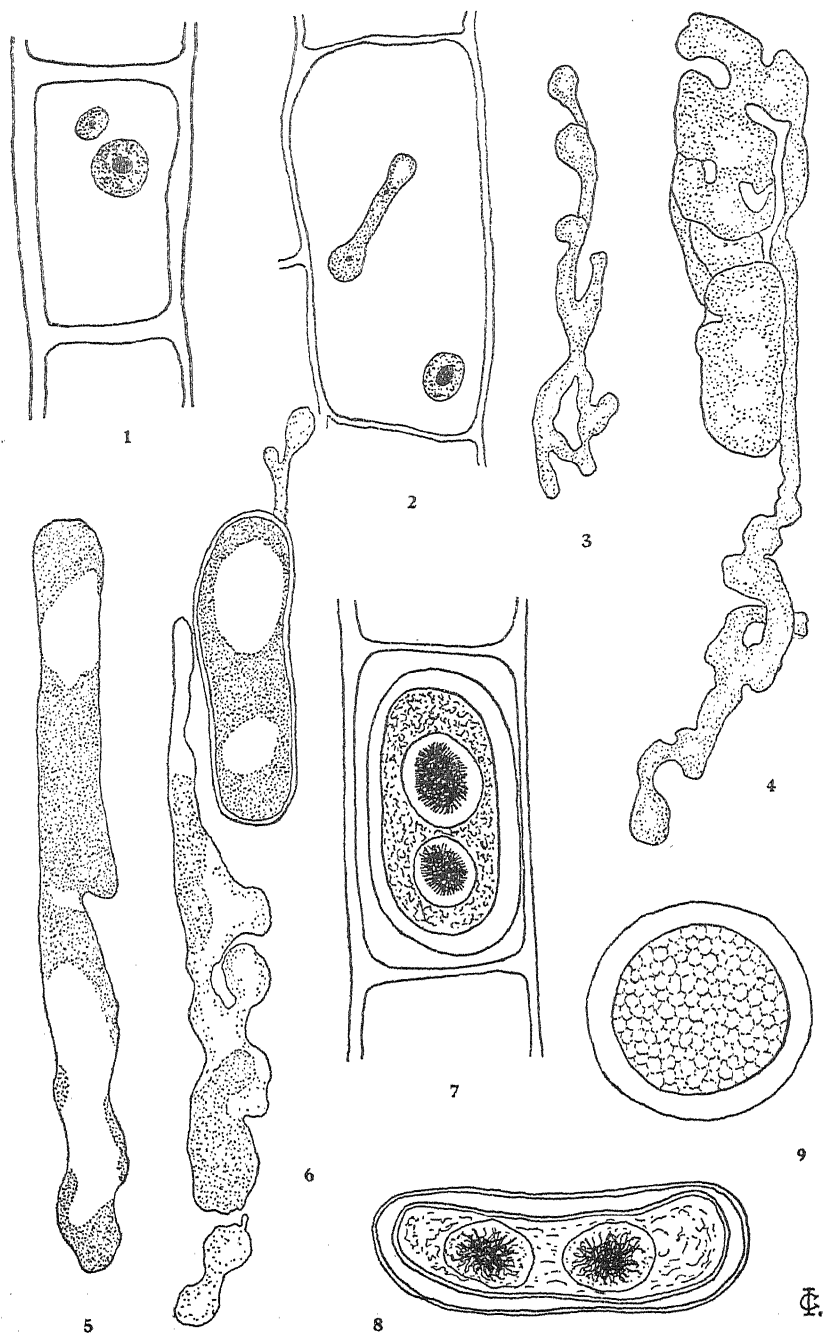
The organism at present under investigation differs from *C. graminis* Büsgen both in the shape and size of the sporangia and in the colour of

the walls. (Taking the average of the examples from Kew and neglecting the material from Surrey, the average sporangium size is  $33.9 \times 30.1 \mu$  as against  $30.7 \times 14.8 \mu$  in the new material.) It also differs from *P. Agrostidis* in the size and colour of the spores.

On the other hand, the size and shape of the resting sporangia agree with those of *C. caespitis*, since both elongated and spherical sporangia were found by Griffon and Maublanc and both types appear in the present material. It also agrees in the very pale yellow or almost colourless wall. It seems therefore justifiable to refer the organism now recorded as attacking seedlings of *Agrostis* to that species, and to suggest that the material from Surrey, preserved in the Kew herbarium as *C. graminis*, is also *C. caespitis*. So far as can be ascertained, *C. caespitis* has not been recorded in this country by any other worker.

#### LIFE-HISTORY OF THE ORGANISM.

A study of the life-history of the organism has confirmed the account given by Griffon and Maublanc (2). Infection has not been observed, but it is concluded that it takes place by means of zoospores which enter the host cell and form a small spherical body (Text-fig. 1). This gradually becomes elongated and forms a short mycelial hypha (Text-fig. 2), which then branches, forming an irregular mass of hyphae in the host cell (Text-fig. 3). The content of these hyphae is densely granular, and although apparently multinuclear, few details of their structure could be made out; no septa are formed. As the hyphae grow they become swollen at various points, forming rounded or irregular vesicles in which the protoplasm may become vacuolated (Text-fig. 4). In some instances the whole of a hypha may swell (Text-fig. 6), but more often fine hyphae are developed which pass from cell to cell of the host and travel for a considerable distance before a fresh vesicle is formed. Inside the vesicles the protoplasm becomes more dense, and, when mature, the vesicles are surrounded by a cellulose wall which at first is thin (Text-fig. 7), but subsequently assumes an increasing thickness. These vesicles thus become differentiated into the resting sporangia. Apparently at first they are uninucleated, though in fully mature ones several nuclei can sometimes be made out. After the formation of a resting sporangium the hyphae associated with it disappear rapidly, so that in fully mature sporangia no trace of the original mycelium can be detected (Text-fig. 8). These resting sporangia are usually ellipsoidal (Text-figs. 8-9), though spherical ones are sometimes found (Pl. III, Fig. 1). The wall of the mature sporangium is thick and varies from  $3$  to  $5 \mu$  in width. In colour it is generally of a very slightly yellow tinge; the outer surface is smooth. Within the sporangium oil is stored, first as numerous small droplets, but as the sporangium matures



TEXT-FIGS. 1-9. All figures were made with a camera lucida at table level using a Zeiss 1/12 objective (n.a. 1.40) and compensating ocular  $\times 6$ , giving a magnification of 1140. 1. Young



these run together, forming either one or, in ellipsoidal sporangia, two masses. In stained preparations these have a thread-like appearance (Text-figs. 8 and 9 and Pl. III, Fig. 1).

Griffon and Maublanc, in their description of *C. (Physoderma) caespitis*, record that on one occasion they found, in addition, a single thin-walled zoosporangium which developed zoospores with a single flagellum. No such structures were found in the present material, and it is concluded that they only very rarely appear.

Although attempts were made to germinate the resting sporangia no greater success resulted than was found by Griffon and Maublanc, and it may be possible that something more than climatic conditions are required to achieve this end. It is known that the spores of some fungi germinate only after they have passed through the alimentary tract of an animal. From what has been found in similar species it seems probable that they give rise to a number of zoospores, a character which is suggested by the multinucleated condition of the resting sporangium. There is no evidence, however, that these zoospores fuse in pairs when they escape, thus indicating a condition of sexual reproduction.

#### DISCUSSION.

The systematic position of the species included in the Cladochytriaceae is very unsatisfactory, since it is a matter of disagreement in which of three genera a particular species should be placed. Mention has been made of the fact that *C. graminis* and *C. (Physoderma) caespitis* have been provisionally placed in the genus *Cladochytrium*, yet the obviously closely allied *P. Agrostidis* has been separated into another genus. It should be explained, however, that von Minden (7) transferred *C. graminis* also to the genus *Physoderma*. According to the original description of the genus *Cladochytrium*, as given by Nowakowski (8), the mycelium of the fungus always gives rise to a zoosporangium from which uniflagellate zoospores are produced. Such a condition has been described by Nowakowski (8) in *C. tenue*, and it is also true of the less well-known *C. polystomum*, described by Zopf (12). In neither of these species are resting sporangia known. In *C. Nowakowskii*, recently described by Sparrow (10), the mode of reproduction is normally by zoosporangia, but in a single instance a resting sporangium with a thick wall was observed,

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protoplast shortly after infection of the host-cell. The larger of the two bodies is the host-cell nucleus. 2. Young mycelium developing in a host-cell. 3. Mycelium showing the development of branches and secondary filaments and the commencement of swellings of the mycelium. 4. Mature mycelium showing the swellings becoming differentiated into resting sporangia. 5. Mycelium in which the whole is swollen, showing the gradual separation and contraction of the cytoplasm. 6. Mycelium with fully formed resting sporangia. 7. Resting sporangium in a host-cell. 8. Resting sporangium showing the striated thickening sometimes appearing on the wall. 9. Spherical resting sporangium.

showing that two types of sporangia could be developed. *C. caespitis* of Griffon and Maublanc can also produce both types of sporangia, only here the thick-walled type is the normal and the thin-walled the exception. It will be seen therefore that it is impossible to segregate into two genera those which reproduce only by zoosporangia and those which reproduce only by resting sporangia without encountering intermediate forms which appear to indicate that the one condition has originated from the other.

The genus *Physoderma* is separated from the genus *Cladochytrium* by the fact that in the former genus resting sporangia only are produced, although in *P. maculare* and *P. butomi* zoosporangia have been recorded, albeit not derived directly from the mycelium. In view of the rare appearance of zoosporangia in *C. caespitis* it is quite likely that these structures also occur occasionally in several of the species now included in the genus *Physoderma*. In any case, under the present system *C. caespitis* obviously should not be placed in the genus *Physoderma* since it produces zoosporangia; at the same time *C. graminis* and *C. Agrostidis* must be relegated to the genus *Physoderma* because, having been less closely investigated, no zoosporangia have been discovered, despite the fact that the three species are so much alike that only by small characters like the size of the resting sporangia can they be separated from one another. It seems likely, from a study of the various species which have been described, that the production of zoosporangia depends largely upon the conditions under which the fungus is growing. In those species which live in water plants there is a tendency to produce zoosporangia, especially in those parts of the plant which are in contact with the water, while on the other hand, in plants where there is less moisture, resting sporangia become the common mode of reproduction.

When the genus *Urophlyctis* was erected by Schroeter (9) the problem became more complex. Schroeter considered that the turbinate enlargements from which the resting sporangia develop constituted a sexual process, resulting in the formation of a sexually produced sporangium. In this view he was supported by Magnus in a series of papers (4), (5), and (6). This view has not been supported by more recent studies of the species, and few workers now would regard the occurrence of sexuality in *Urophlyctis* as in any way probable. If, then, the resting sporangia eventually arise from the mycelium without any sexual process there is nothing to separate that genus from the genus *Physoderma*. We have, therefore, evidence that the three genera, *Cladochytrium*, *Physoderma*, and *Urophlyctis*, constitute an evolutionary series which cannot satisfactorily be split up. At the same time it is obvious that there is a marked difference between *C. tenue* and *Urophlyctis Alfalfae*. Von Minden (7) got over the difficulty of separating the genera by rather artificial distinctions. In the genus *Cladochytrium* he placed only those forms in which reproduction

was by zoosporangia. He used the genus *Physoderma* for those species which reproduce by resting sporangia, but which cause only slight swelling of the host tissue; while he relegated to the genus *Urophlyctis* those species in which typical hypertrophy was produced. This is obviously an unnatural classification, since, for example, *U. pulposa* possesses both zoosporangia and resting sporangia.

It has been suggested that there is no evidence to show that the thick-walled structure characteristic of these fungi finally produces a number of zoospores, and that it should therefore be called a sporangium. As a matter of fact many books speak of it as a resting spore. One of the most critical investigations on this point was made by Tisdale (11), who found that the resting sporangium opened by a lid, through which numerous unflagellate zoospores escaped. He studied their development, but obtained no evidence of any sexual fusion. It is unfortunate that the germination of these resting sporangia has been found so difficult to bring about in most species, as further light on their development might show that they were not all sporangia. At the same time, their general similarity in size and structure certainly suggests that they represent a similar organ in all the species.

Fischer placed all the species known to him in the single genus *Cladochytrium*, distributing them among three sub-genera, *Cladosporangium*, *Physoderma*, and *Urophlyctis*. Despite the more recent elevation of these sub-genera to full generic rank it would seem that Fischer's treatment is the most satisfactory. It is suggested that, using the same features which were employed by von Minden for distinguishing the three genera, these three sub-genera may be separated from one another without thereby splitting up the species so drastically as has been done by von Minden. Thus all species which ever produce resting sporangia should be included in the sub-genus *Physoderma*, and the sub-genus *Cladosporangium* should include only *C. tenue* and possibly *C. polystomum* and *C. cornutum*. On the other hand, in view of the small number of species which would be so included in this sub-genus, it might be more satisfactory to drop the sub-genus *Physoderma* and include all these species in the sub-genus *Cladosporangium*, leaving the sub-genus *Urophlyctis* for those species in which definite hypertrophy is apparent. In this way the close sequence between *C. tenue* and such a form as *C. caespitis* would not be interrupted since they would all belong to the same sub-genus. Whatever the ultimate method may be whereby the classification of these fungi is effected, it must be recognized that the one at present adopted is both unsatisfactory and unscientific.

## SUMMARY.

*Cladochytrium caespitis* Griffon and Maublanc is described from this country for the first time. The life-history has been followed and is found to agree with the previous account.

Evidence is brought forward to show how this species differs from *C. graminis* Büsgen and *C. Agrostidis* Lag., and it is suggested that the organism described by Massee as *C. graminis* was probably *C. caespitis*. In this case there appears to be no record of either *C. graminis* or *C. Agrostidis* in this country.

The classification of the three genera *Cladochytrium*, *Physoderma*, and *Urophlyctis* is discussed. It is urged that it would be better to revert to Fischer's treatment and regard them all as sub-genera of *Cladochytrium*, and that the two sub-genera *Cladosporium* and *Physoderma* should be combined, since a series of types passing from one sub-genus to the other can be traced.

DEPARTMENT OF BOTANY,  
THE UNIVERSITY, BRISTOL.  
February, 1933.

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EXPLANATION OF PLATE III.

Illustrating Dr. Ivey Cook's paper on 'Some Observations on the Genus *Cladochytrium*'.

All figures magnified 300 times.

Fig. 1. Photomicrograph of the sporangia of *Cladochytrium graminis* Büsgen from the leaf of *Dactylis glomerata* in the Kew herbarium.

Fig. 2. Photomicrograph of the sporangia of *Physoderma Agrostidis* Lag. from a leaf of *Agrostis alba* in the British Museum herbarium.

Fig. 3. Photomicrograph of the sporangia of *Cladochytrium* (*Physoderma*) *caespitis* Griffon and Maublanc from a root of *Agrostis* sp. from the Yorkshire material.



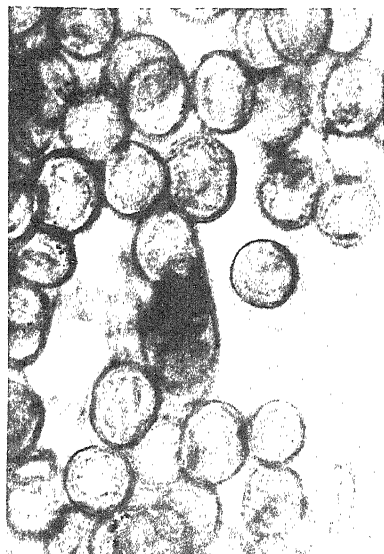


Fig. 1.

*C. graminis.*

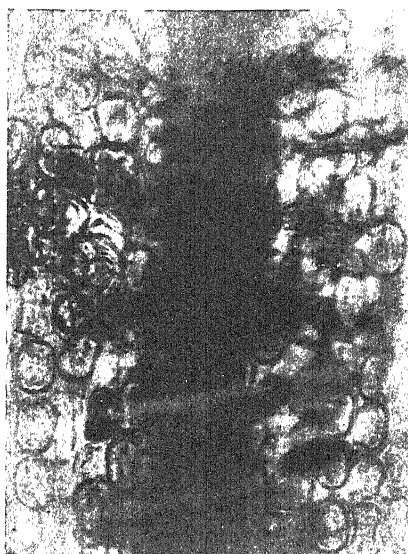


Fig. 2.

*C. Agrostidis.*



Fig. 3.

*C. caespitis.*

W. R. I. C. photo.

Huth coll.

IVIMEY COOK — CLADOCHYTRIUM.





# Studies in the Physiology of Parasitism.

## XIV. Comparison of Enzymic Extracts Obtained from Various Parasitic Fungi.<sup>1</sup>

BY

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With twenty-eight Figures in the Text.

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### I. INTRODUCTORY.

IT is now well known that in the mechanism of parasitism an important part is played by a cell-wall dissolving enzyme—pectinase—secreted by the hyphae of the parasite. The further questions arise as to whether

<sup>1</sup> Thesis submitted for Ph.D. of London University, Oct. 1932.

parasitic fungi secrete such an enzyme over a range of nutrient conditions, and whether the enzyme obtainable from one fungus agrees in properties with that obtained from another. This investigation deals chiefly with the latter problem, though the former is incidentally touched upon.

## II. HISTORICAL.

The earlier literature dealing with the secretion of pectinase ('cytase' of older papers) by various plant pathogenic fungi and bacteria has been summarized by Brown (1) in an earlier number of this series. It is only necessary therefore to review the relevant publications which have appeared since that date.

In a later paper by Brown (2) it was shown that active preparations of the pectinase enzyme could be obtained not only from the fungal germ-tubes, but from the medium in which these had grown, and that in fact such preparations of exo-enzyme, though relatively impure, were the most active that had so far been obtained. With the method of preparation adopted, the enzymic activity reached a maximum within a few days of spore sowing, after which it diminished.

Harter and Weimer (4) working with *Rhizopus tritici* were able to demonstrate the presence of pectinase both in the mycelial extracts of the fungus and also in the decoction of sweet potato in which they grew it. They found that the activity of the endo-enzyme was greatest on the first and second days of growth, while that of the exo-enzyme had a broad maximum extending from the second to the seventh day.

A comparison of the pectinase produced by different species of *Rhizopus* was made by them. Eleven species of the fungus were used and all of these were found to produce the enzyme and to exude some of it into the culture solution. The amount of pectinase produced varied with different species. Two species *nigricans* and *artocarpi* secreted relatively small amounts of pectinase while *chinensis* and *microsporus*, two non-parasitic species, secreted a comparatively large quantity of the enzyme into the culture solution.

In a later paper, Harter and Weimer (5) examined the influence of the substrate and of its pH concentration on pectinase production. They grew *Rhizopus* on various plant decoctions and synthetic media. It was found that pectinase was produced on all vegetable media except prune juice, but not on a synthetic solution where glucose was the source of carbon. When pectin was substituted for glucose in Czapek's Solution an active enzyme was produced. With sweet potato decoction the enzyme was produced at all the different pH concentrations tried whereas Czapek's Solution over a wide range of pH concentration gave no evidence of enzyme secretion. Hence they concluded that the composition of the

medium influences the production of pectinase while its pH concentration has no effect.

Muhleman (7) has given evidence of the production of pectinase in *Sclerotinia cinerea*. He was able to demonstrate it in the watery extracts of the fungal mycelium but not in the medium (prune juice) in which he grew the fungus. He obtained the best results after 3, 4, or 5 days of fungal growth.

Paul (8) demonstrated the presence of an exo- and endo-enzyme in a number of strains of *Botrytis cinerea* and was unable to correlate the enzymic activities shown with the known differences in virulence of the strains. In this respect he confirmed the findings of Harter and Weimer.

Willaman and Davison (9), in an investigation of the pectic constituents of the plant cell-wall, examined the pectin-dissolving properties of certain fungi, more particularly those of a species of *Rhizopus*. They divided the group of enzymes which affect pectin substances into three, viz. :

(1) *Protopectinase* (= 'pectosinase' of older nomenclature) which decomposes the insoluble protopectin present in the cell-walls, possibly in a loose combination with cellulose. The hydrolytic product is soluble pectin.

(2) *Pectase*, which converts pectin into a gel, pectic acid.

(3) *Pectinase* (in narrow sense) which hydrolyses pectin and pectic acid into their simple soluble cleavage products, arabinose, galactose, &c.

As no one has yet examined biochemically the end products of the cell-wall action of *B. cinerea* it cannot be stated that all three enzymes cited above are present in extracts of this fungus. From the plant pathological point of view it is clear that it is the first enzyme that is of prime importance. Therefore in speaking of the pectinase of the fungi used in this work, it will be understood that it is the protopectinase of Willaman and Davison's classification that is indicated.

Recently a comparative study of the pectinase enzyme of a number of fungi was carried out by Chona (3). The fungi used were *B. cinerea*, *Fusarium fructigenum*, *Phytophthora erythroseptica*, and *Pythium de Baryanum*. The first two of these normally attack apple and the last two potato tissue. The enzymes of *Botrytis* and *Fusarium* were obtained from the ground-up germ-tubes according to Brown's method ; those of the potato-attacking fungi by growing the latter on sterilized, living potato tissue and squeezing out the juice from rotted portions. In all cases the enzymic solutions were used in the crude form.

Chona tested these enzymes in regard to two properties, (a) dependence of activity on pH, (b) the relative retarding effects of plant juices on activity. He found that the curve of activity of *Botrytis* enzyme in relation to pH showed a steep downward gradient on the alkaline side, though not so near to the neutral point as Brown had found. In contrast to this

behaviour the enzyme of *Pythium* was found to increase in activity on the alkaline side.

The retarding effect of plant juices on enzymic activity was studied with special reference to potato and apple juices. Chona found that the enzymes of *B. cinerea* and *F. fructigenum* were more sensitive to concentrations of potato juice than of apple juice and that the converse applied as regards the enzymes of *P. erythroseptica* and *P. de Baryanum*. The specific retarding factor in apple juice was found to be its acidity; that of potato juice to be its content of certain salt radicles, especially magnesium and phosphate.

Chona's work therefore suggested at least two types of pectinase (strictly protopectinase) enzyme, viz., that possessed by the potato-attacking fungi, characterized by sensitiveness to acidity and relative insensitiveness to certain salts, and that possessed by apple-attacking fungi characterized by the converse properties.

The object of the writer's study was to examine the conclusions which had been tentatively put forward by Chona. For this purpose a number of other fungi were introduced into this investigation. An important point to notice is that the enzymes used by Chona were of two sorts as regards method of preparation. In the case of the apple-attacking fungi he used solutions of the endo-enzyme as extracted from germ-tubes, whereas the preparations from *Pythium* and *Phytophthora* were exo-enzymic in nature. The latter preparations also were obviously highly impure. It was possible, therefore, that the results obtained by Chona might be due in part at least, not to the different fungi as such, but to the different methods of preparation or to different amounts of impurities involved. Accordingly, an attempt was made in this work to compare the enzymes from the various fungi used on a common basis, at least in the matter of preparation.

### III. EXPERIMENTAL METHODS.

#### (a) *Fungi used.*

The list of fungi used in this work was as follows:

Parasitic on apple:

*Botrytis cinerea.*

*Monilia fructigena.*

*Gloeosporium fructigenum.*

*Fusarium fructigenum*, C<sub>21</sub> strain of Brown.

Parasitic on potato:

*Pythium de Baryanum.*

*Phytophthora erythroseptica.*

All these fungi were obtained from the stock cultures kept in the Plant Pathology Laboratory of the Imperial College of Science and Technology, London. Pure cultures were maintained and repeatedly sub-cultured on slopes of potato extract agar in boiling tubes incubated at 20° C.

(b) *Preparation of Crude Enzymic Extracts.*

Two methods of preparation were adopted, viz., the plate method of Brown in which the fungi were germinated in liquid extracts, and a plug method where pieces of plant tissue were inoculated and subsequently extracted. The range of media employed was as follows:

*Turnip juice*, obtained by steaming peeled turnips cut up into small pieces, without addition of water, for half an hour; subsequently straining off the liquid through fine muslin and autoclaving for half an hour at 15 lb. pressure.

*Potato juice*. The method used for turnip extract is inapplicable to potato as the gelatinization of the contained starch makes the extraction of the juice very difficult. Peeled raw tubers were passed through a mincing machine and the juice then expressed through fine muslin. The liquid so obtained which contains a large amount of starch in suspension was cleared in the centrifuge. The cleared liquid cannot be steamed or autoclaved without formation of a copious sticky precipitate. As it was proposed to use an extract approximating as closely as possible to the natural sap of potato, such heat sterilization methods were inadmissible. The following compromise was adopted.

The cleared liquid was kept for 24 hours in an ice chest, after which it was heated for a short time to 60° C. and then returned to the ice chest. This process was repeated three times. The liquid thus treated was much more slowly overrun with bacteria when used for the preparation of the enzyme than when merely kept in the ice chest. The three short heat treatments were not sufficient to cause any noticeable coagulation of the protein contents.

*Synthetic medium* of the following composition:

Glucose	25	gram.
Asparagin	10	"
KH <sub>2</sub> PO <sub>4</sub>	5	"
MgSO <sub>4</sub>	2.5	"
Water	1,000	c.c.

This medium was sterilized in the autoclave in the usual way. Other media used were plugs of potato, apple, and turnip.

The details of preparation of the crude extract for each fungus will now be described.

(i) *Botrytis cinerea*. A dense spore suspension of the fungus was

made by adding 0.1 c.c. wet volume of the spores to 10 c.c. of the nutrient liquid. This suspension was sown on circular glass plates that had been sterilized by autoclaving and laid horizontally one on top of the other on small porcelain insulators. The tier of plates thus formed was set in a large flat-bottomed dish containing water so as to check evaporation, and the whole covered by a large bell jar. After 48 hours' growth the mass of germ-tubes was scraped off and the fluid of germination separated by filtration through muslin. This liquid, which contains a strong solution of the enzyme was in general used straight away, but in some cases the active principle was purified and collected as will be described later.

The mass of germ-tubes was placed in a muslin bag, washed well first in running tap-water and afterwards in sterile distilled water to remove any trace of nutrient that might be present. The water from the gelatinous mass of germ-tubes thus collected was squeezed out as much as possible and the whole mycelial mat spread on a plate and dried *in vacuo* over calcium chloride in a desiccator. The dry mycelium was scraped off with a knife, mixed with an equal weight of sand and ground to a fine powder. The extract from this powder was obtained by digesting it in distilled water in the proportion of 0.2 grm. to 3 c.c. of water. The sand and fungal debris from the extract were removed by centrifuging. The media used in this connexion were potato and turnip extracts and the synthetic solution.

The method of using plugs of plant tissue was adopted by Chona in the preparation of the enzymes of *Pythium* and *Phytophthora* from potato tissue. He attempted to keep the conditions of growth as natural as possible by using unheated tissue. With this object in view he sterilized the potatoes externally by steeping the peeled tubers in mercuric chloride solution for 20 minutes and then washing the traces of the latter off in sterile distilled water. The washed tubers were placed in sterilized jam jars having some moist absorbent cotton wool at the bottom to maintain a humid atmosphere for the growth of the fungus. The mouths of the jam jars were closed with the lids of Petri dishes. The tubers were then inoculated with the fungi and incubated at 20° C. for a week. After this time it was found that a good portion of the potato was rendered soft by the fungal invasion. The rotted portions of the tubers were scraped off with a knife, collected in a muslin bag, and the juice extracted under slight pressure. This juice was tested for enzymic activity and found to be quite active.

In practice this method proved to be very troublesome, as whole batches were spoiled from time to time by bacterial contamination. As the method of surface sterilization appeared to be sufficiently drastic, it may be seriously doubted whether the contaminating bacteria were not already present within the tissue of the potatoes used, and were able to develop under the conditions of the experiment. Some preparations were,

however, successfully carried out with living material in the manner indicated, but the problem was much simplified when it was found that the same type of result was got by the use of heat-sterilized tissue. For this purpose pieces of potato tissue about 1 inch thick and 2 inches long were placed in large plugged boiling tubes ( $1\frac{1}{2}'' \times 7''$ ) on top of a wad of moist cotton wool and autoclaved. Plugs of apple and turnip were similarly used. The inoculated plugs were incubated at 20° C. for ten days, by which time it was found that they were completely covered with fungal growth. The juice of the infected tissue was now extracted in the usual way, cleared of spores and mycelial debris by centrifuging, and purified by the method to be described later.

(ii) *Monilia fructigena*. Earlier workers have reported failure to obtain the pectinase enzyme of this fungus. Willaman and Davison worked with *Monilia fructigena* and *M. cinerea*, and to obtain the enzyme grew them in culture flasks on prune juice. The mycelia were harvested after two days, dried and ground, and an extract made by digesting the powder in water. It was found that the fungal extract did not show any indication of the presence of the enzyme. The medium in which the fungi had been growing was also tested for enzymic activity, but here also the results were disappointing.

Willaman and Davison next used Brown's method of sowing thick spore suspensions of the fungi in a nutrient medium in flask cultures and allowing them to germinate for just 24 hours. After this time the mycelial growths were treated as before, but the extracts so obtained did not show any pectinase activity.

To obtain the enzyme from *Monilia* in the course of the present work the fungus was grown in flask cultures on different media, e.g. extracts of potato, apple, and turnip, oatmeal porridge, Richards's and Brown's solutions. Pectinase activity was tested for both in the mycelial extracts and in the liquids in which the fungi had been growing. In all these cases the results were negative.

The failure of Willaman and Davison to obtain the enzyme was probably due to the fact that the time allowed for the germination of these fungi was too short. Brown in his enzymic work used *B. cinerea*, which, being a rapidly growing fungus produced in 24 hours enough mycelial germ-tubes to secrete the enzyme copiously. As will be seen later in this work it has been possible to obtain the pectinase from *Monilia* by Brown's method. It was found that there was not enough mycelial growth, even after 48 hours, and therefore the time allowed for germination was lengthened to five days.

The cultures of *Monilia* used for the preparation of spores in quantity were set up on potato mush agar acidified with a trace of malic acid and incubated at 20° C. The details of preparation of the crude enzymic

solutions are identical with those for *Botrytis* except that the time allowed for growth of germ-tubes was five days. This rather lengthy germination period made it impossible to use potato extract as nutrient, since this medium is too favourable to bacterial growth. The medium used was turnip extract.

This fungus was also grown on autoclaved plugs of potato, apple, and turnip, and extracts prepared as described for *Botrytis*.

(iii) *Pythium de Baryanum* and *Phytophthora erythroseptica*. In the case of these two potato-attacking fungi, Brown's method of sowing thick spore suspensions on glass plates could not be employed on account of the relatively few spores produced. Other methods were tried. Culture flasks containing different media were inoculated with the fungi and incubated at 20° C. The various media tried were potato extract, potato mush, turnip extract, oatmeal extract, Richards's solution, and Brown's solution. Both the fungi were found to grow well on all the media used. The mycelial growths were harvested at various intervals of time and pectinase activity was tested for, both in the watery extract of the dried mycelia and in the media in which the fungi had been growing. In all these cases there was no evidence of the presence of an enzyme.

Active extracts were finally obtained by growing these fungi on plugs of sterilized potato tissue and expressing the juice after the manner described above.

(iv) *Fusarium fructigenum* and *Gloeosporium fructigenum*. The enzymes from these two fungi were prepared by the plug method, and in the case of the former also, by the plate method of Brown. Turnip plugs were used and the crude enzymes were collected in the usual way.

#### (c) *Purification of Enzymic Extracts.*

The various enzymic extracts prepared as described above are obviously of different degrees of impurity, and some of them contain relatively large amounts of substances derived from the host tissues. Some degree of purification was therefore called for. The method adopted was the following :

The crude enzymic solution, in whatever way prepared, was first cleared of matter in suspension by centrifuging, then added to three times its own volume of 95 per cent. alcohol. The precipitate formed was separated by centrifuging, transferred to a glass plate and dried *in vacuo* over calcium chloride in a desiccator. The dried precipitate was scraped off the glass plate, mixed with an equal weight of silver sand, and ground to a fine powder in a mortar. For experimental purposes 0.5 grm. of this powder was added to 20 c.c. of distilled water and the sand from the solution removed by centrifuging.

Though it will appear probable from results to be described later that



some important impurities have not been removed by the process of precipitation with alcohol, it is certain that a large amount of crystalloidal alcohol-soluble impurity is thereby eliminated. Repetition of the process with a view to further purification was not attempted in view of the laborious method of preparation entailed and of the losses of active enzyme which follow from attempts at purification. All the enzymic preparations used were subjected to one purification, and to no more.

(d) *Quantitative Comparison of Enzymic Extracts.*

In the absence of a purely chemical test based upon the hydrolysis of a pure pectin substrate, Brown's mechanical test method was adopted. This consists in determining the time required for the loss of coherence to take place when discs of plant tissue of standard thickness are immersed in the enzymic solution.

For this purpose a cylinder of approximately 1.5 cm. in diameter is cut from the plant tissue (e.g. potato tuber), placed for half an hour in water to become completely rigid, then cut into discs of 0.5 mm. thickness on a hand microtome. These discs after being placed in the enzymic solution are examined at frequent intervals by slight pulling. The end point of the reaction is when discs can be torn without sensible mechanical resistance. As a standard test three discs were placed in each 2 c.c. of the enzymic solution.

The tissues used were potato, turnip, and apple. Potato was much the most useful, as suitable tubers could be obtained all the year round. Turnip tissue is only suitable when the roots are fairly young, and relatively few apple varieties can be used as the majority of those obtainable in the shops have tissues too soft or too crumbly for this purpose.

In any particular comparison it is essential that the test-discs be all cut from the same tuber as there is considerable variation from one tuber to another as regards the rate of enzymic digestion.

(e) *Determination of H-ion Concentration.*

Enzymic extracts of different acidity or alkalinity were prepared by the careful addition of decinormal HCl or NaOH. Control series were set up by making similar additions to enzymic solutions which had been deactivated by boiling. The range of pH concentrations over which tests could be made was limited by the fact that high H-ion or OH-ion concentrations both have in themselves a macerating effect on plant tissue.

The pH concentration of a solution so made was first approximately determined by a spotting method, using the B.D.H. Universal Indicator. When the pH value was thereby roughly fixed, 1 c.c. of the solution was diluted to 10 c.c. with distilled water and a standard quantity of the appropriate indicator added. The pH was determined to within 0.2 of a

unit by comparing the colour given by the indicator with a series of standard coloured charts (Clark).

(f) *Dilution of Enzymic Extracts with Plant Juices.*

The plant juices used were those of potato, apple, and turnip. For this purpose the plant tissue was cut into small pieces, wrapped in unsized filter-cloth, and the juice squeezed out under high pressure in a mechanical press. The turbid liquid so obtained was cleared as far as possible by centrifuging and used in making a standard series of dilutions of the enzymic extract. The effect of mere dilution on the activity of the extract was determined by running a control series in which the diluent was pure water.

#### IV. EXPERIMENTAL RESULTS.

(a) *The Effect of H-ion Concentration on the Activity of Enzymic Extract.*

A range of pH concentrations was set up by adding 0.1, 0.3, 0.5, and 0.7 c.c. of N/10 HCl and N/10 NaOH to each 4 c.c. of enzymic solution. In order to control any hydrolytic effect due to the acid or alkali concentration itself a parallel series was set up in which the active enzyme was replaced by enzyme deactivated by boiling. Two c.c. of each solution was removed and used for the determination of the pH values, to the remainder were added the test discs of tissue and the time required for disintegration noted. The inverse of this time is taken as a measure of enzymic activity. As a standard for comparison the activities are reduced to a basis of 100 for the solution to which neither acid nor alkali has been added. Table I gives an illustrative set of figures for the enzyme of *B. cinerea* as prepared by extracting germ-tubes grown in turnip extract. The test tissue used was potato.

TABLE I.

Preparation	Active enzyme.			Deactivated enzyme.	
	pH.	Time.	Activity.	pH.	Time.
4 c.c. + 0.7 c.c. N/10 HCl	2.6	0.8 hrs.	237	2.8	3.5 hrs.
" + 0.5 " "	3.8	1.0 "	190	4.0	>24 "
" + 0.3 " "	5.0	1.5 "	127	5.2	" "
" + 0.1 " "	5.4	1.8 "	106	5.6	" "
" + 0.1 c.c. N/10 NaOH	6.6	1.9 "	100	6.8	" "
" + 0.3 " "	7.2	2.3 "	83	7.4	" "
" + 0.5 " "	8.2	4.0 "	47.5	8.4	" "
" + 0.7 " "	9.4	8.0 "	23.75	9.4	5 "
" + 0.7 " "	9.8	8-17 "		9.8	2.8 "

Within the limits of pH 3.8-8.2 there was no appreciable maceration

due to the acid or alkali added, and the effect observed can therefore be ascribed to the action of the enzyme.

As a general control to these experiments a series of solutions of different pH was set up by adding various amounts of decinormal acid or alkali to water (with 0.1 M glycocoll added as a buffer), and testing the range within which there was no appreciable maceration caused by the chemical. In various tests, both with potato and and turnip tissues, it was found that the limits within which decomposition of the discs could be definitely ascribed to the enzyme were 3.1–3.5 to 9.0–9.4. For the sake of safety all parts of the curves outside the range 3.5–9.0 are disregarded.

Figs. 1–6 illustrate the effect of pH upon the activity of *Botrytis* enzyme prepared in a variety of ways.

The general shape of all these curves is the same, viz., a fall from the acid to the alkaline side. The rather steep rises shown in some of the graphs at the acid end may in part be due to incipient hydrolysis by the acid, and should be interpreted conservatively.

In each of Figs. 1–6, and in all subsequent figures, unless otherwise stated, the activity of the enzymic solution to which neither acid nor alkali is added is taken as 100. The absolute activities are therefore not comparable as from one figure to another. It will be noted that the pH of the purified enzyme, indicated by the ordinate, varies somewhat, lying usually between pH 6–7, but that in the preparation from apple plugs (Fig. 6) it is distinctly higher. This difference presumably indicates incomplete purification.

Figs. 7–10 illustrate the behaviour of the enzyme of *M. fructigena*. A comparison of Figs. 7 and 8 shows that, even when the fungus is grown on the same medium, preparations of exo-enzyme and endo-enzyme behave differently. Figs. 9 and 10, while agreeing with each other, show enzymic solutions which behave differently from those of Figs. 7 and 8. This is all the more remarkable as Figs. 8 and 9 both refer to the exo-enzyme of *Monilia* prepared from turnip media, viz., turnip juice in the former and turnip tissue in the latter.

A corresponding curve was prepared for the exo-enzyme of *Monilia* as grown on potato plugs. This was very similar to the curves of Figs. 9 and 10.

A further series of graphs for the four remaining fungi examined is shown in Figs. 11–16.

All these curves are of the same type as shown for *M. fructigena* under the same conditions.

The only references bearing on this subject are those by Brown (1), Willaman and Davison (9), and Chona (3). Though Brown expressed his results in terms of titrable value and not of pH, it is clear that he found an optimum enzymic activity in the neighbourhood of the neutral point, activity falling off gently towards the acid side and very sharply on

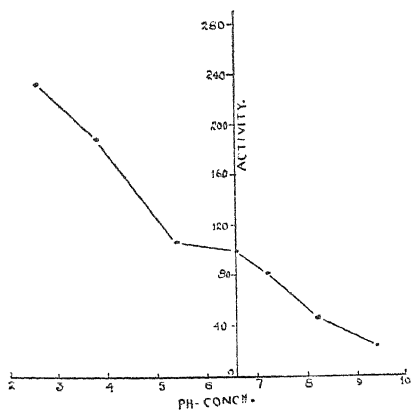


FIG. 1.

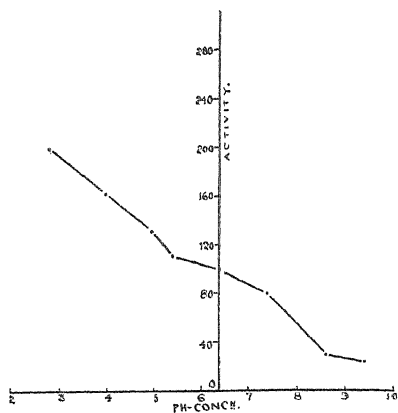


FIG. 2.

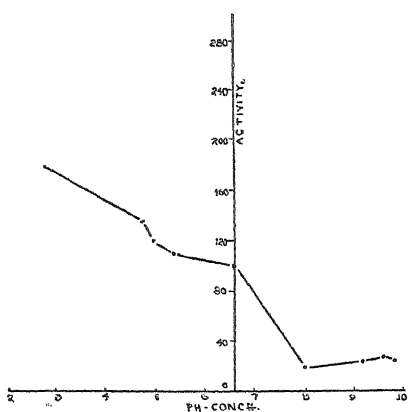


FIG. 3.

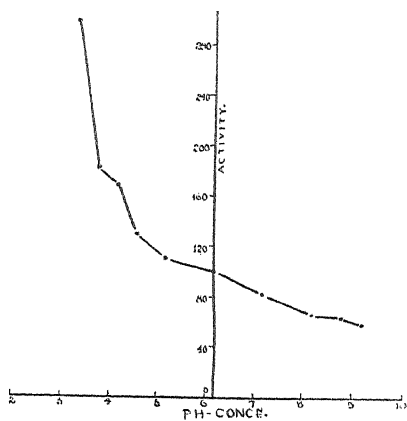


FIG. 4.

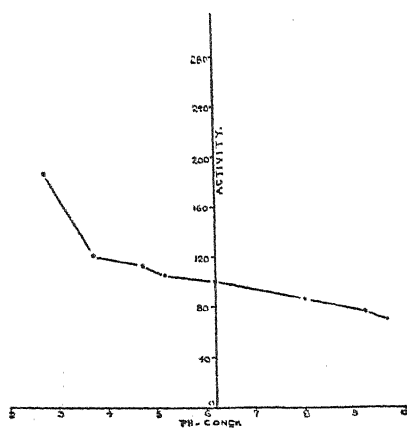


FIG. 5.

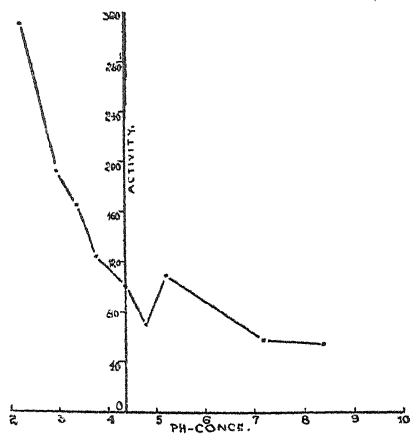


FIG. 6.

FIGS. 1-6. Effect of pH on activity of *Botrytis cinerea* enzyme. Fig. 1. Endo-enzyme, spores germinated in turnip juice. Fig. 2. Endo-enzyme, spores germinated in potato juice. Fig. 3. Exo-enzyme, spores germinated in turnip juice. Fig. 4. Exo-enzyme, fungus grown on turnip plugs. Fig. 5. Exo-enzyme, fungus grown on potato plugs. Fig. 6. Exo-enzyme, fungus grown on apple plugs.

the alkaline side. Willaman and Davison found an optimum point for the activity of the enzyme of *Rhizopus tritici* in the neighbourhood of pH 5.

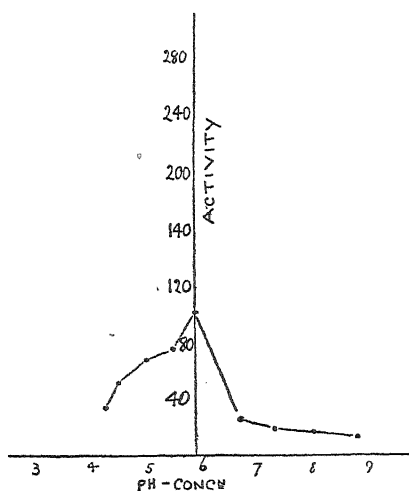


FIG. 7.

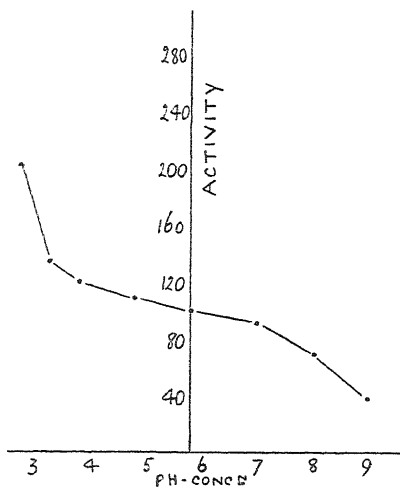


FIG. 8.

FIGS. 7 and 8. Effect of pH on activity of *Monilia fructigena* enzyme prepared from spores germinating on turnip juice. Fig. 7. Endo-enzyme. Fig. 8. Exo-enzyme.

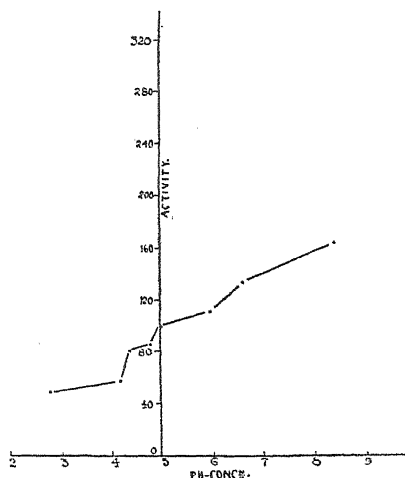


FIG. 9.

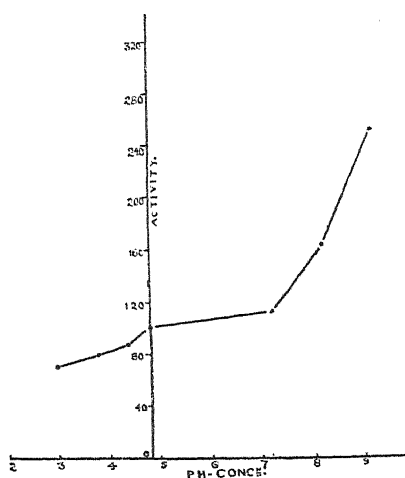


FIG. 10.

FIGS. 9 and 10. Effect of pH on activity of *Monilia fructigena* enzyme. Fig. 9. Exo-enzyme from turnip plugs. Fig. 10. Exo-enzyme from apple plugs.

The results given by Chona for the internal unpurified enzyme of *Botrytis* prepared from spores grown on potato juice and for the external unpurified enzyme of *Pythium* prepared from invaded potato plugs agree respectively with Figs. 2 and 14 of this paper.

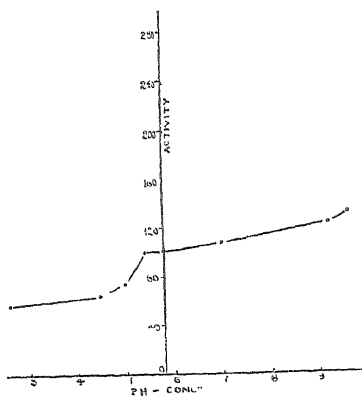


FIG. 11.

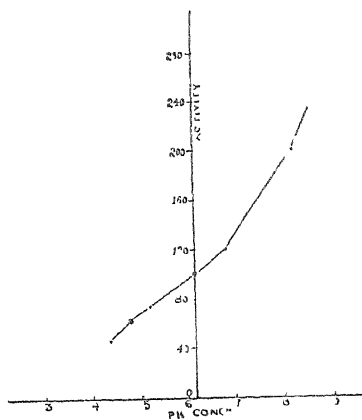


FIG. 12.

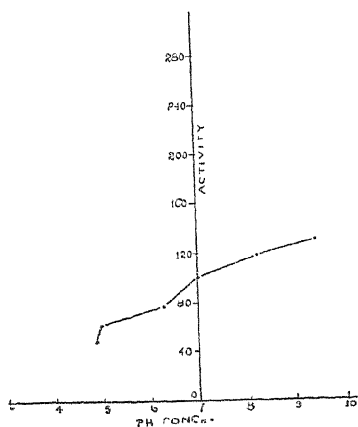


FIG. 13.

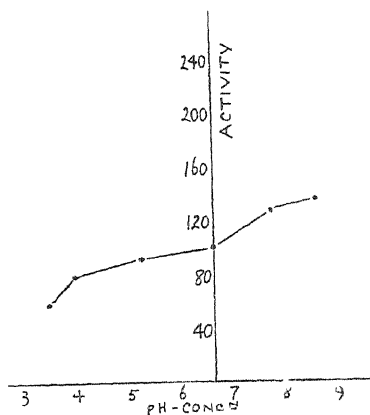


FIG. 14.

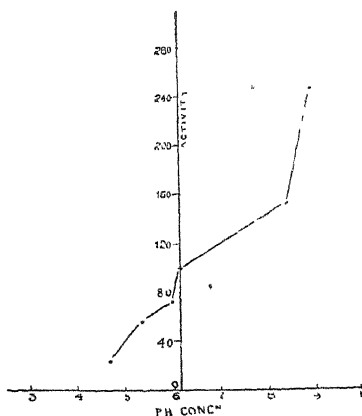


FIG. 15.

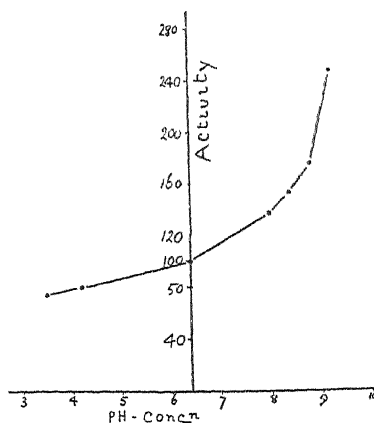
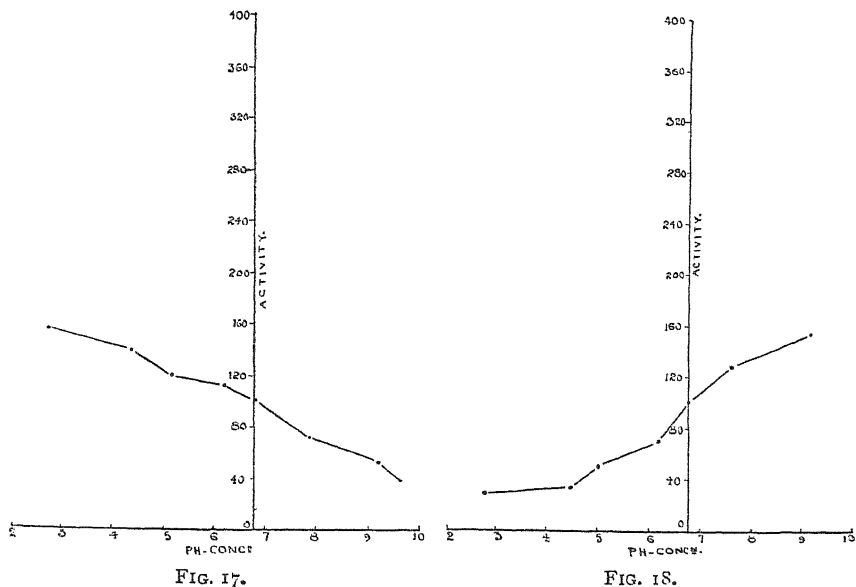


FIG. 16.

FIGS. 11-16. Effect of pH on activity of exo-enzyme of various fungi on various media. Fig. 11. *Fusarium fructigenum* grown on autoclaved turnip plugs. Fig. 12. *Gloeosporium fructigenum*, as in Fig. 11. Figs. 13 and 14. *Pythium de Baryanum*, grown on autoclaved and fresh potato plugs, respectively. Figs. 15 and 16. *Phytophthora erythroseptica*, as in Figs. 13 and 14, respectively.

It is clear, therefore, that there is very considerable variation in the results which have been obtained. A consideration of the different results obtained by Brown and the writer for the enzyme of *B. cinerea* suggests



FIGS. 17 and 18. Effect of pH concentration on activity of mixed enzymic preparations. Fig. 17. Active *Botrytis* enzyme + equal volume of deactivated *Pythium* enzyme. Fig. 18. Active *Pythium* enzyme + equal volume of deactivated *Botrytis* enzyme.

that different strains of the same fungus may vary in that respect. This type of result is not without parallel in enzymic literature, e.g. in the different affinity constants shown by the invertase enzyme prepared from different yeast races by Kuhn (6).

That the enzyme from the same fungus does not always behave in the same way is illustrated by the behaviour of the preparations from *Monilia fructigena* (Figs. 7-10). This result is perhaps best explained on the grounds that the effect of pH on enzymic activity is conditioned to some extent by the presence of accessory substances or impurities. That impurities are still present in the preparations which have been subjected to one precipitation by alcohol is indicated by a certain amount of variation in the pH of the standard preparations, as already noted (p. 197). One is led to suspect therefore that the process adopted only gives partial purification, and that certain impurities which remain considerably modify the behaviour of the enzyme. As a parallel illustration may be cited the observation of Willstätter and co-workers (10) who found that the optimum pH for the lipase of the stomach was markedly affected by the degree of its purity.

As there is evidence that there are impurities which cannot be removed

by precipitation with alcohol, an attempt was made to circumvent their action by the following procedure.

Instead of using the enzyme from *B. cinerea*, a mixture was made by the addition of an equal quantity of the enzyme from *Pythium* deactivated by boiling. A similar preparation was made from *Pythium* enzyme by the addition of an equal quantity of deactivated *Botrytis* enzyme. The impurities of the two enzymes are thus divided on a common basis, assuming of course that these impurities are not destroyed or altered in properties by boiling. The relation of pH to enzymic activity was tested in those mixtures. Figs. 17 and 18 show the results obtained.

Fig. 17 is similar in shape to Figs. 1 and 2 which refer to *Botrytis* enzyme alone, and Fig. 18 is similar to Figs. 13 and 14 which refer to *Pythium* enzyme alone. It thus appears that when one ensures that as far as possible the same impurities are present, the enzymes of the two fungi behave differently. The suggestion to this effect put forward by Chona is thus fully supported.

(b) *Retarding Effect of Plant Juices on Enzymic Activity.*

It was found by Chona that the internal enzyme from the apple-attacking fungus, *B. cinerea*, was more sensitive to potato juice than to apple juice, while the external enzyme from *Pythium* which is a potato-attacking fungus was more sensitive to apple juice. The work which will be described in this section is an extension along these lines.

Starting from the original enzymic extracts, a series of dilutions was set up—full strength, 80, 60, 50, 30, 20, and 10 per cent.—the diluting liquids being water and various plant juices of full strength prepared as described previously. The activities of these enzymic dilutions were determined on standard tissue discs as usual. An illustrative set of figures giving the time in hours necessary for loss of coherence is contained in Table II. The enzyme used in this case was the internal enzyme from *B. cinerea* obtained by growing the spores on turnip juice extract.

TABLE II.

Diluting liquid.	100 %.	80 %.	60 %.	50 %.	30 %.	20 %.	10 %.
Water	1.5	2.0	2.5	3.25	3.5	3.8	4.5
Potato juice	"	5.8	20	> 24	> 24	> 24	> 24
Apple juice	"	4.6	4.8	5.6	6.5	7.5	21
Turnip juice	"	2.0	4.5	5.0	6.0	6.5	7.0

On taking the inverse of these times as a measure of activity and giving the activity of the undiluted enzyme a value of 100, one obtains the graph shown in Fig. 19. Figs. 19–24 are a representative set of such graphs.

All the data obtained in this connexion are summarized in Table III.



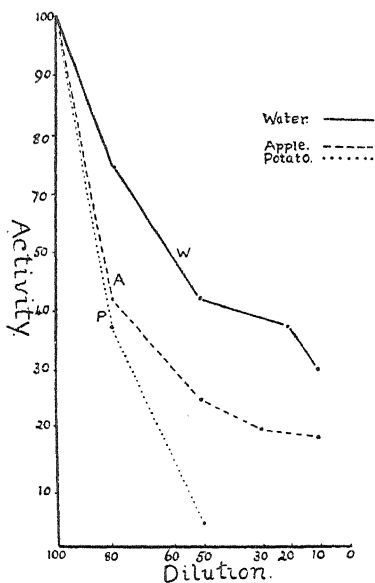


FIG. 19.

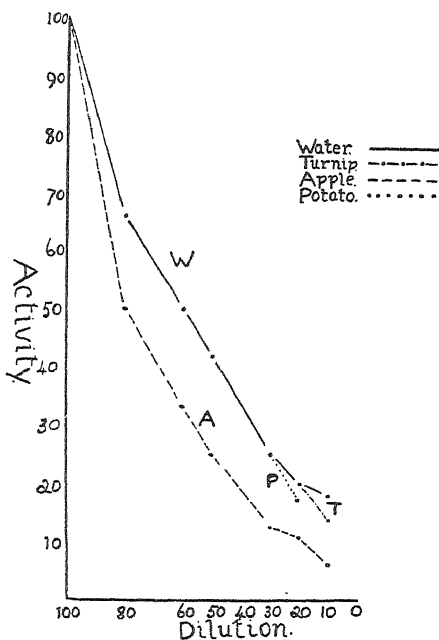


FIG. 20.

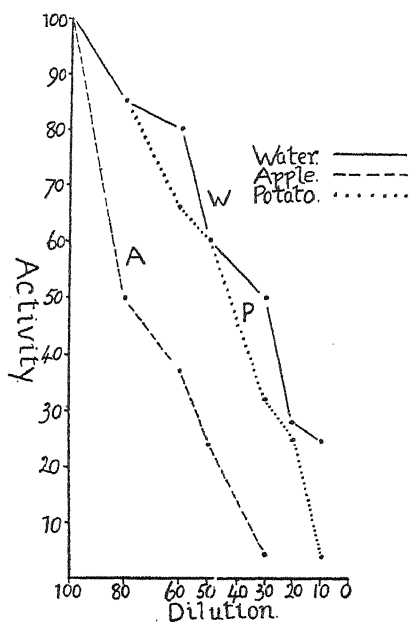


FIG. 21.

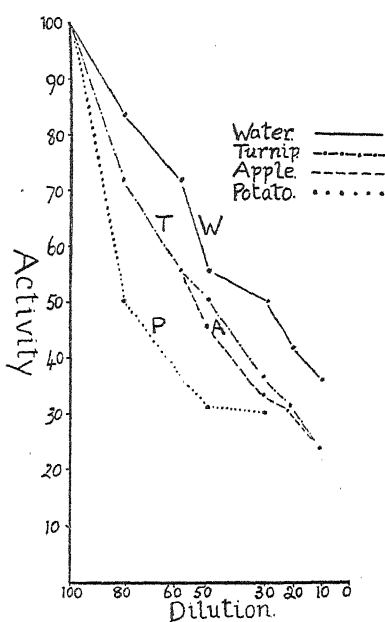


FIG. 22.

FIGS. 19-22. Effect of dilution with water (W), and potato (P), apple (A), and turnip (T) juices on enzymic activity. Fig. 19. *Botrytis* endo-enzyme, prepared from potato extract. Fig. 20. *Botrytis* exo-enzyme, prepared from potato extract. Fig. 21. *Botrytis* exo-enzyme, prepared from potato plugs. Fig. 22. *Botrytis* exo-enzyme, prepared from apple plugs.

The notation used in the last column of the table will be understood from the following example: by  $P > A \geq T = W$  is meant that—

(1) Potato juice has a distinctly greater retarding action than apple juice.

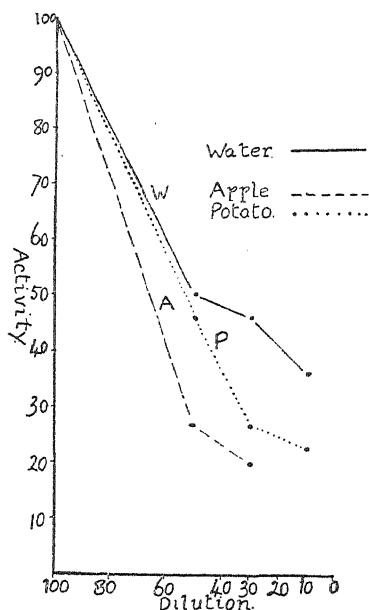


FIG. 23.

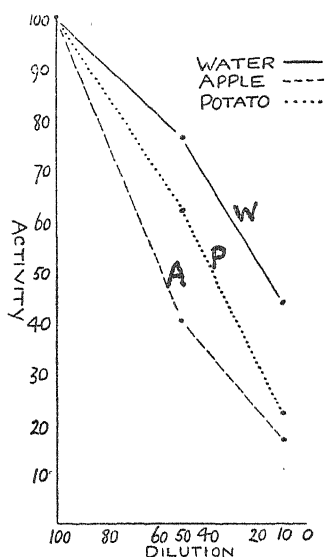


FIG. 24.

FIGS. 23, 24. Effect of dilution with water (W), and potato (P), and apple (A) juices on enzymic activity. Fig. 23. *Pylhium* exo-enzyme, prepared from potato plugs. Fig. 24. *Phyto-phthiura* exo-enzyme, prepared from potato plugs.

(2) Apple juice has a slightly greater retarding action than turnip juice.

(3) Turnip juice does not differ from water in its action.

The results of Table III are derived from experiments carried out at all seasons of the year, and therefore with plant material which varied considerably in composition. In spite of this, however, a good degree of regularity in the results is shown. Though complete data for some of the fungi are not available, there is a clear suggestion from Table III that the external enzyme prepared on a particular medium is less sensitive to that medium than to another. This conclusion comes out most distinctly when one confines attention to exo-enzyme prepared on apple or potato media and tested with apple or potato juices, as shown in Table IV which is abstracted from the preceding Table.

The preparations of endo-enzyme need not be considered in this connexion, partly because the data are so few, and partly because it is the behaviour of the exo-enzyme that it is obviously of chief interest.

TABLE III.

No.	Fungus.	Enzyme.	Nutrient medium.	Retarding effect.
1.	<i>Botrytis</i>	Endo	T. extract	P>A>T>W
2.	"	"	" "	P>A >W
3.	"	"	P. "	P>A >W
4.	"	Exo	Synthetic	P>A ≧W
5.	"	"	" "	P>A ≧W
6.	"	"	T. extract	P≧A ≧T>W
7.	"	"	" "	P>A >W
8.	"	"	P. "	A>P ≧T≧W
9.	"	"	" "	A>P >W
10.	"	"	T. plug	A>P=T>W
11.	"	"	" "	A>P >W
12.	"	"	P. "	A>P ≧W
13.	"	"	A. "	P>A=T>W
14.	"	"	" "	P>A =W
15.	<i>Monilia</i>	Endo	T. extract	A≧P >W
16.	"	Exo	" "	A>P=T>W
17.	"	"	P. plug	A>P >W
18.	"	"	T. "	A>P ≧T≧W
19.	"	"	" "	A>P >W
20.	"	"	A. "	P≧A >W
21.	<i>Fusarium</i>	"	T. "	A>P=T>W
22.	<i>Gloeosporium</i>	"	" "	A>T>P>W
23.	"	"	" "	A>P >W
24.	<i>Pythium</i>	"	P. "	A>P >W
25.	"	"	" "	A>P W
26.	<i>Phytophthora</i>	"	" "	A>P=T ≧W

TABLE IV.

No. in Table III.	Fungus.	Medium.	Retarding effect.
8 and 9	<i>Botrytis</i>	P. extract	A>P
12	"	P. plug	A>P
13 and 14	"	A. "	P>A
17	<i>Monilia</i>	P. "	A>P
20	"	A. "	P≧A
24 and 25	<i>Pythium</i>	P. "	A>P
26	<i>Phytophthora</i> .	P. "	A>P

Potato and apple are presumably the most dissimilar of the three plant media used, and so the contrast 'Potato v. Apple' is more clean cut than either of the contrasts 'Potato v. Turnip' or 'Turnip v. Apple'. Nevertheless the data regarding the two latter comparisons indicate the same general conclusion. Compare, for example, nos. 10 and 13 of Table III as regards 'Turnip v. Apple' and nos. 6, 8, and 10 of Table III as regards 'Turnip v. Potato'. It appears that the retarding action of turnip juice tends to be less than that of potato or apple juice, but that when the enzyme is prepared on media other than turnip, the retarding action of turnip juice is considerably enhanced.

Just as was shown in the section dealing with the effect of pH concentration on activity, so also it appears here that some substances associated with the enzymes in accordance with the manner of their preparation

The notation used in the last column of the table will be understood from the following example: by  $P > A \geq T = W$  is meant that—

(1) Potato juice has a distinctly greater retarding action than apple juice.

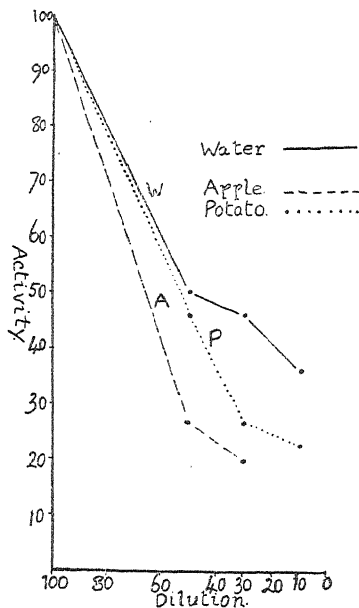


FIG. 23.

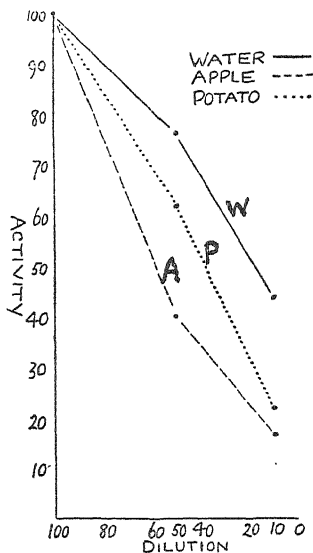


FIG. 24.

FIGS. 23, 24. Effect of dilution with water (W), and potato (P), and apple (A) juices on enzymic activity. Fig. 23. *Pythium* exo-enzyme, prepared from potato plugs. Fig. 24. *Phytophthora* exo-enzyme, prepared from potato plugs.

(2) Apple juice has a slightly greater retarding action than turnip juice.

(3) Turnip juice does not differ from water in its action.

The results of Table III are derived from experiments carried out at all seasons of the year, and therefore with plant material which varied considerably in composition. In spite of this, however, a good degree of regularity in the results is shown. Though complete data for some of the fungi are not available, there is a clear suggestion from Table III that the external enzyme prepared on a particular medium is less sensitive to that medium than to another. This conclusion comes out most distinctly when one confines attention to exo-enzyme prepared on apple or potato media and tested with apple or potato juices, as shown in Table IV which is abstracted from the preceding Table.

The preparations of endo-enzyme need not be considered in this connexion, partly because the data are so few, and partly because it is the behaviour of the exo-enzyme that it is obviously of chief interest.

TABLE III.

No.	Fungus.	Enzyme.	Nutrient medium.	Retarding effect.
1.	<i>Botrytis</i>	Endo	T. extract	$P > A > T > W$
2.	"	"	" "	$P > A > W$
3.	"	"	P. "	$P > A > W$
4.	"	Exo	Synthetic	$P > A \approx W$
5.	"	"	"	$P > A \approx W$
6.	"	"	T. extract	$P \approx A \approx T > W$
7.	"	"	" "	$P > A > W$
8.	"	"	P. "	$A > P \approx T \approx W$
9.	"	"	" "	$A > P > W$
10.	"	"	T. plug	$A > P = T > W$
11.	"	"	" "	$A > P > W$
12.	"	"	P. "	$A > P \approx W$
13.	"	"	A. "	$P > A = T > W$
14.	"	"	" "	$P > A = W$
15.	<i>Monilia</i>	Endo	T. extract	$A \approx P > W$
16.	"	Exo	" "	$A > P = T > W$
17.	"	"	P. plug	$A > P > W$
18.	"	"	T. "	$A > P \approx T \approx W$
19.	"	"	" "	$A > P > W$
20.	"	"	A. "	$P \approx A > W$
21.	<i>Fusarium</i>	"	T. "	$A > P = T > W$
22.	<i>Gloeosporium</i>	"	" "	$A > T > P > W$
23.	"	"	" "	$A > P > W$
24.	<i>Pythium</i>	"	P. "	$A > P > W$
25.	"	"	" "	$A > P > W$
26.	<i>Phytophthora</i>	"	" "	$A > P = T \approx W$

TABLE IV.

No. in Table III.	Fungus.	Medium.	Retarding effect.
8 and 9	<i>Botrytis</i>	P. extract	$A > P$
12	"	P. plug	$A > P$
13 and 14	"	A. "	$P > A$
17	<i>Monilia</i>	P. "	$A > P$
20	"	A. "	$P \approx A$
24 and 25	<i>Pythium</i>	P. "	$A > P$
26	<i>Phytophthora</i>	P. "	$A > P$

Potato and apple are presumably the most dissimilar of the three plant media used, and so the contrast 'Potato v. Apple' is more clean cut than either of the contrasts 'Potato v. Turnip' or 'Turnip v. Apple'. Nevertheless the data regarding the two latter comparisons indicate the same general conclusion. Compare, for example, nos. 10 and 13 of Table III as regards 'Turnip v. Apple' and nos. 6, 8, and 10 of Table III as regards 'Turnip v. Potato'. It appears that the retarding action of turnip juice tends to be less than that of potato or apple juice, but that when the enzyme is prepared on media other than turnip, the retarding action of turnip juice is considerably enhanced.

Just as was shown in the section dealing with the effect of pH concentration on activity, so also it appears here that some substances associated with the enzymes in accordance with the manner of their preparation

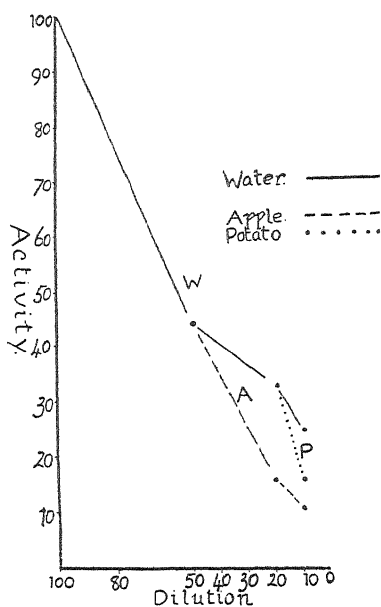


FIG. 25.

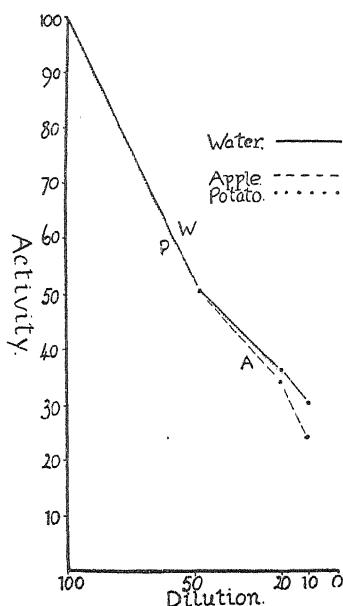


FIG. 26.

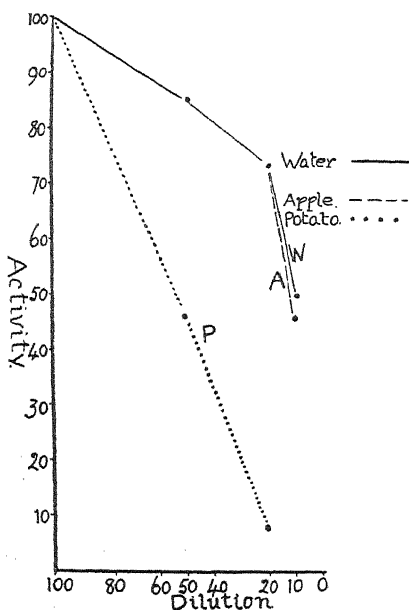


FIG. 27.

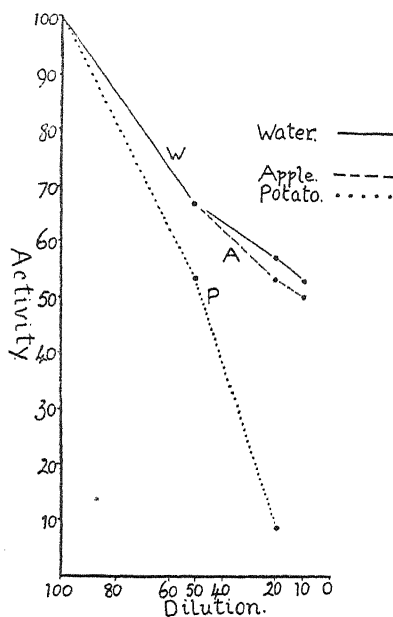


FIG. 28.

FIGS. 25-8. Effect of dilution on enzymic activity (*Botrytis cinerea*). Fig 25. Exo-enzyme from turnip. Fig. 26. Exo-enzyme from turnip + deactivated enzyme from apple. Fig. 27. Exo-enzyme from apple. Fig. 28. Exo-enzyme from apple + deactivated enzyme from turnip.

influence the behaviour of the enzymes in the presence of retarding agents. These associated substances are not removed, at least entirely, by a single precipitation with alcohol. An experiment was therefore carried out to see if the same kind of difference would be shown if the impurities are approximately the same in both cases. The method adopted was the same as already described (p. 202).

The crude external enzyme of *Botrytis* was extracted in the usual way from cultures on turnip and apple plugs and the following preparations tested against potato and apple juice:

- (i) *Botrytis* enzyme from turnip tissue (Bt).
- (ii) " " " apple " (Ba).
- (iii) Bt + deactivated Ba, in equal proportions.
- (iv) Ba + " Bt " " "

Figs. 25–8 illustrate the effects of dilution with various media on the activities of these four preparations.

It appears therefore that the enzyme of *B. cinerea* prepared from apple plugs shows the same features whether used alone or mixed with the deactivated extract from turnip; and similarly for the enzyme prepared from turnip plugs. This result is similar to that already described (p. 202). The further consideration of this point will be given in the next section.

In analysing the retarding effects of potato and apple juices on enzymic activity Chona found that the specific action of potato juice was due chiefly to its content of magnesium sulphate and potassium phosphate. As we have seen that the sensitiveness of the pectinase enzyme to various plant juices varies according to the manner of preparation, one would anticipate that Chona's conclusions regarding these particular salts would also stand in need of revision. Experiment has shown that this is the case.

Table V shows how equal concentrations of  $\text{MgSO}_4$  and  $\text{K}_3\text{PO}_4$  affect the activity of the external and internal enzymes of *Botrytis* prepared from cultures on turnip juice.

TABLE V.

Enzyme.	Diluent.	100 %.	50 %.	25 %.
Endo	Water	100	71	43
	0.4 % $\text{MgSO}_4$	"	0	0
	0.6 % $\text{K}_3\text{PO}_4$	"	0	0
Exo	Water	100	75	50
	0.4 % $\text{MgSO}_4$	"	50	33
	0.6 % $\text{K}_3\text{PO}_4$	"	33	0

It is clear that both salts exert a retarding action, but that the latter is much greater in the case of the endo- than of the exo-enzyme.

## V. DISCUSSION.

It will be remembered that Chona, working with the endo-enzyme of *Botrytis* and the exo-enzyme of *Pythium*, found that there was a marked difference between them in acid relationships and in tolerance of certain chemicals and plant juices. He was led to consider that there were at least two types of pectinase enzyme. The data presented here point to another conclusion. While Chona's results have been fully confirmed, it has been found that the same differences of behaviour noted by him as between the enzymes of *Botrytis* and *Pythium* may be shown by enzymic preparations of the same fungus according to the method of preparation. It is more rational therefore to postulate the same enzyme in all cases and to ascribe the differences observed to accompanying substances. Such substances might be constituents of the medium in which the fungus has grown, or metabolic products arising in the course of growth. In that case there would be no difficulty in understanding why the same fungus, when grown on different media, should give enzymic preparations which acted differently, or why the enzymic preparations of different fungi grown on the same medium might be different. The pectinase could quite well be the same in all cases, but its action would be conditioned by a different set of substances in each case.

As the enzymic preparations purified by alcoholic precipitation still show the differences in question, it is clear that the modifying substances are not easily removed from the enzyme, and one is led to suspect adsorption compounds. The results obtained with mixed enzymic preparations (pp. 202 and 207) would then be interpreted on the assumption that the 'accompanying substances' are likewise deactivated by heating or that the enzyme when saturated with one set of substances can no longer adsorb another set.

It is obvious that the results of this paper render the interpretation of the phenomenon of parasitism on an enzymological basis more complex. The experimental technique can, however, probably be simplified in such a way as to lead to further results. The specific retarding substances are of quite simple nature, viz., acids or salts, and one is not therefore compelled to work with complicated decoctions like apple and potato extracts. It seems probable that the same kind of differences could be produced by suitable modifications of a single synthetic medium. It would be interesting, for example, to compare the enzyme secreted by *B. cinerea* on a medium of high magnesium sulphate or acid content with that formed in a medium low in these ingredients.

Not merely do the properties of the enzyme depend upon the type of medium used, but there is also evidence that the latter may determine whether any enzyme is formed at all. It was noted (p. 194) that cultures



of *Pythium* and *Phytophthora* when grown in various decoctions produced no enzyme either within the hyphae or in the nutrient liquid, whereas the same fungi when grown on plant tissue secreted the enzyme freely. A somewhat similar result was found for *M. fructigena*. Similar conclusions were reached by Robin (Thesis for Diploma of Imperial College, 1931) who found that the enzymes of certain plant pathogenic bacteria were readily obtained from cultures on plant tissue but not so readily from decoctions, even of the same plant tissue. One is thus led to suspect that the presence of the substrate pectin exerts a favourable influence on the secretion by parasitic organisms of the enzyme pectinase. Harter and Weimer's (5) statement that *R. tritici* forms no pectinase when grown in Czapek's solution but does so when soluble pectin is substituted for glucose in that solution would fall in line with this hypothesis.

This work was carried out at the suggestion and under the direction of Prof. W. Brown, to whom I wish to express here my thanks.

## VI. SUMMARY.

1. The pectinase enzyme has been extracted from the following fungi: *B. cinerea*, *M. fructigena*, *G. fructigenum*, *F. fructigenum*, *P. de Baryanum*, and *P. erythroseptica*, under a variety of nutrient conditions.

2. The enzymic preparations have been tested from the point of view of (a) effect of pH concentration on activity, (b) specific retarding action of various plant extracts and chemicals.

3. From these tests it appears that the nutrient medium influences to a considerable extent the precise behaviour of the enzyme of any particular fungus.

4. The results obtained are best interpreted on the assumption that the enzyme is the same in all cases, but that certain of its properties are profoundly modified by the adsorption of substances from the nutrient medium.

5. The capacity of fungi to secrete pectinase also depends on the medium. In particular it is suggested that the presence of pectin in the substrate favourably affects the secretion of pectinase enzyme.

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# The Cytology and Morphology of *Neurospora tetrasperma* Dodge.

BY

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With Plates IV and V and six Figures and two Diagrams in the Text.

## INTRODUCTION.

*NEUROSPORA TETRASPERMA* is one of the species of a new genus established by Dodge (10) in 1927. In that paper he described his collection of fungi of the *Monilia sitophila* group, 'the Red Bread Moulds'. Some of these possessed a perithecial stage, and were placed by him in a new genus *Neurospora* containing four species.

In *N. sitophila* and *N. crassa* the ascus contains eight initially uninucleate spores, the mycelia produced from these are of two kinds, both species being constantly heterothallic. In *N. tetrasperma* and *N. erythraea*, the asci usually contain only four spores. In the case of *N. tetrasperma*, which was the more fully investigated, these spores are initially binucleate. The mycelia produced from these spores are homothallic. Occasionally more than four spores are to be found in an ascus, smaller and initially uninucleate spores being present in addition to initially binucleate spores. The mycelia produced from the small spores are heterothallic.

In a second paper in the same year Dodge (11) suggested that in spite of the apparently homothallic condition of *N. tetrasperma* there must occur a segregation of factors capable of determining heterothallism in one of the three nuclear divisions in the ascus. He put forward three alternative schemes of nuclear behaviour in the ascus to account for this.

Dodge's schemes of nuclear behaviour in the ascus were, to some extent, speculative, and the present investigation of the cytology and morphology of *N. tetrasperma* was undertaken in order to ascertain which scheme is supported by fact. There were also other possible points of interest, for instance, the number of nuclear fusions and subsequent reduction divisions to be found in the life-history of a Pyrenomycetous fungus. It seemed that independent work on a member of a group of fungi, which

had not been studied cytologically, would be of use in clarifying the position with regard to the nuclear behaviour among the Ascomycetes.

I am indebted to Dr. Dodge for his gift of cultures which made the present investigation possible.

#### MATERIAL AND METHODS.

The cultures sent to me by Dr. Dodge in 1930 have been maintained since that date on a maize agar. A decoction is prepared by boiling 25 grm. of maize, grinding the softened grains, reboiling and straining off the solid matter. The liquid is then made up to a litre, 3 per cent. agar is added and the whole is sterilized. The original cultures obtained from Dodge were two of the single strains from small spores labelled by him 'Race S<sub>6</sub> Haplont A' and 'Race S<sub>1</sub> Haplont B'; and a culture of the two strains growing in contact and bearing perithecia. The two single strains are referred to throughout this paper as A and B respectively. When A and B are growing together they give cultures which are, to all intents and purposes, like the original homothallic strain, and normal (i.e. initially binucleate) spores from them when grown singly give homothallic cultures. The cultures throughout the work were not grown in incubators and were therefore subjected to the ordinary fluctuations of room temperature. It was found that the time required for the development of perithecia varied with the temperature and might be anything from eight to twenty days.

Fixations were made at all ages in several fixatives. Great difficulty was encountered at first in obtaining well-fixed material of the older perithecia. The perithecial wall is thick and only slightly permeable, the central cavity of the perithecium contains air and penetration is therefore slow. The cytoplasm of the asci and the nuclei were often much contracted in consequence. This difficulty was largely overcome by cooling the fixative before use in iced water (2°–6° C.) or by immersing the block of agar bearing the perithecia in iced water for half a minute before fixing. Flemming's strong fluid diluted with an equal volume of water and La Cour's 2BD (29) proved to be the most satisfactory fixatives.

Material taken up through absolute alcohol and chloroform and embedded at once proved to be more satisfactory than material which was stored in Calberla's fluid before embedding. The asci in the stored material became slightly swollen thereby causing an exaggerated appearance of vacuolation in the cytoplasm, while the cytoplasm itself showed a tendency to hold haematoxylin more than in freshly fixed specimens.

Microtome sections were cut from 6–12  $\mu$  thick and dried thoroughly on a water bath for five days. The sections were mordanted in 8 per cent. iron alum, stained in haematoxylin (Heidenhain's) and destained in 4 per cent. iron alum acidified with acetic acid.<sup>1</sup> Some of the preparations

<sup>1</sup> 1 drop of glacial acetic acid to 30 cc. of 4 per cent. alum.

were counterstained with erythrosin in clove oil, others were left without a counterstain.

In addition to sections, whole mounts of archicarps and young perithecia were made. These were stained with erythrosin in 50 per cent. glycerine and mounted in glycerine jelly (25).

The small size of the chromosomes made the use of critical illumination of the first importance, and throughout the work an oil immersion condenser also was necessary; this greatly facilitates the use of the higher powered compensating oculars.

#### DEVELOPMENT OF THE PERITHECIUM.

Moreau and Moreau (30) in 1930 very briefly described some of the early stages of the development of the perithecium. It will be seen that the following account, based on the examination of abundant material, differs from theirs in several important details.

Archicarps of identical appearance are initiated in the cultures of A and B strains both when they are grown separately and when A and B are in contact (Pl. IV, Figs. 1-3). These archicarps are small coiled multinucleate hyphae filled with densely granular cytoplasm. They consist of more than one cell, but the cells are very long like those of the vegetative hyphae and in the young stages often only one cross wall is present (Pl. IV, Fig. 1). The archicarp is said by Moreau and Moreau to consist of a basal region and a terminal trichogyne. This, however, seems to be a purely artificial distinction, for there does not appear to be any morphological difference between the base and the tip of the coil (Pl. IV, Fig. 4).

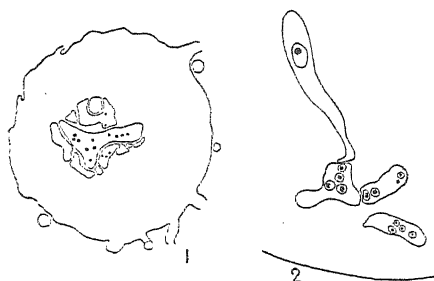
Antheridia have not been seen in any of the numerous cultures examined.

The archicarps appear simultaneously in the three types of cultures and soon become enveloped in sheaths of sterile hyphae which grow up from their bases. The time necessary to attain this state of development, as already mentioned, varies considerably with the temperature.

The development of the archicarps is arrested after the production of the sheaths in the cultures where either A or B strain is growing alone. The primordia then remain as small masses of hyphae which may become dark in colour and are the bulbils or sclerotia mentioned but not examined by Dodge (10). Any of these sclerotia if picked off and planted singly in a Petri dish will produce a new growth from the sheath hyphae. The mycelium obtained by this method shows the same A or B character as the parent culture and will, in its turn, produce archicarps. Thus the abortive archicarps act as accessory reproductive bodies.

The primordium continues its development beyond the sclerotial stage if the two strains are growing in contact. It may now be called the young perithecium, and as such, it increases in size and must be studied in section.

The central fertile region is easily distinguishable from the sheath by the possession of granular cytoplasm and larger nuclei (Text-fig. 1, and Pl. IV, Fig. 6). The fertile region extends through several sections and more than



TEXT-FIGS. 1-2. 1. Section of a young perithecium showing multinucleate condition of the central fertile region. The outline of the perithecium is drawn, the hyphae of the wall have been omitted.  $\times 600$ . 2. Portion of the basal region of a mature perithecium showing a young ascus in conjunction with multinucleate cells.  $\times 490$ .

one nucleus can be seen in every cut portion of the hyphae. The multinucleate condition persists throughout development, even up to the production of the asci (Text-fig. 2, and Pl. IV, Figs. 6 and 7). This part of the description is not in agreement with the earlier one by Moreau and Moreau who state that, as development proceeds, the cells of the fertile region become uninucleate. Cross walls are rare and difficult to see, but the cells of the archicarp are certainly multinucleate and remain so throughout.

The sheath hyphae grow rapidly and the fertile region is left at the base of the enlarging perithecium. The branching of the hyphae of the fertile region at this stage becomes so complicated that it is impossible to follow the course of any one hypha even in thick sections. The nuclei are small and it has not been possible to obtain a chromosome count here. In the fertile region no clear distinction can be made between the multinucleate cells and binucleate cells of the 'ascogenous hyphae'. Some apparently binucleate portions can be distinguished; these are probably ascogenous hyphae and not cut portions of multinucleate cells, but it is not possible to be sure of binucleate cells. What is certain is that, from this mass of fertile hyphae, short ascus hooks are produced, each containing two nuclei (Pl. IV, Fig. 8). The only mitotic figures which have been seen here are telophases of the division in the ascus hook. From these it has not been possible to obtain a chromosome count. The two nuclei in the ascus hook divide simultaneously and the four daughter nuclei become arranged as in the figure (Pl. IV, Fig. 9). The two nuclei situated in the curve of the ascus hook now fuse (Pl. IV, Figs. 10 and 11). This is the only fusion which has been observed in *N. tetrasperma*. The curved part of the ascus hook elongates and carries up with it the fusion nucleus. This nucleus becomes the definitive nucleus of the ascus.

## CYTOLOGY OF THE ASCUS.

The definitive nucleus consists at first of a clear nuclear area, a large nucleolus and two separate polar masses of chromatin (Pl. V, Fig. 12). These two masses gradually lose their identity as the ascus reaches its full size, the chromatin becoming distributed uniformly over the nuclear area (Pl. V, Fig. 13). Just before its first division the definitive nucleus shows a number of chromatin bodies, some apparently separate, some connected with others by fine chromatin threads. These bodies bear, however, no numerical relationship to the chromosomes (Pl. V, Fig. 13).

*First division.* The chromosomes have been counted first at diakinesis. Here they are twelve in number and are arranged in six pairs. Two pairs of long chromosomes and four pairs of very short ones can be distinguished (Pl. V, Fig. 14). At metaphase twelve chromosomes, still in pairs, become grouped on the spindle (Pl. V, Figs. 15 and 16). Five pairs have been shown in Fig. 15, the sixth pair probably lying immediately under one of the others; all six pairs can be counted in Fig. 16. At early anaphase six single chromosomes can be seen passing to each pole. The long and short chromosomes are again visible; the four long ones are on the lower side of the spindle, in the figure, and the pairs have only just separated, while the short ones are all well separated from their partners (Pl. V, Fig. 17). Reduction division has taken place at this point.

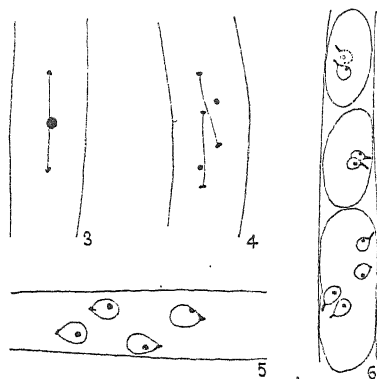
The spindle is long and narrow and intranuclear with deeply staining ends and is nearly always, but not invariably, longitudinally placed in the ascus (Pl. V, Fig. 16). At telophase the spindle is very much elongated and stains deeply with haematoxylin (Text-fig. 3).

*Second division.* In the second division six chromosomes are visible at metaphase in each of the two nuclei in the ascus (Pl. V, Fig. 18). These chromosomes divide and six halves pass to each pole at anaphase (Pl. V, Fig. 19). In Fig. 19 both nuclei in the ascus are in anaphase, ten chromosomes can be counted in one nucleus and twelve in the other; the nuclear area has begun to disappear and the spindle to elongate. This division is thus an equational one and probably represents the homotype.

The behaviour of the spindles has been described by Dodge (11) and my observations are in accordance with his. During the second division the spindles are longitudinally or obliquely placed in the ascus (Text-fig. 4). At telophase they become much attenuated and the daughter nuclei move apart. This movement is so great that sister-nuclei are pushed to opposite ends of the ascus (Text-fig. 5). Non-sister nuclei are therefore adjacent in the ascus during the second interphase.

*Third division.* At the third division six chromosomes appear on the spindle at metaphase and again two long chromosomes and four short ones can be seen (Pl. V, Fig. 20). These divide and six half chromosomes pass

to each pole at anaphase (Pl. V, Figs. 21 *a*, 21 *b*, and 22). Figs. 21 *a* and 21 *b* are two parts of the same ascus cut across between the two pairs of nuclei; in both the lower nuclei the chromosomes can be counted



TEXT-FIGS. 3-6. 3. Telophase of the first division in the ascus showing the elongation of the spindle.  $\times 1,100$ . 4. Telophase of the second division in the ascus showing the elongation of the spindle.  $\times 1,100$ . 5. Rearrangement of the daughter nuclei after the second division showing two daughter-nuclei passing to each end of the ascus; the persistent ends of the spindles give the clue to the arrangement.  $\times 1,100$ . 6. Spore formation after the third division with a pair of non-sister nuclei, marked by the spindle ends, in each spore.  $\times 900$ .

and six are clearly visible at each end of both spindles. This division is therefore also a mitotic one and in *N. tetrasperma* brachymeiosis does not occur.

The description of the behaviour of the spindles at this division is again in agreement with the earlier description by Dodge (11). The spindles are here always obliquely placed, two at each end of the ascus (Pl. V, Figs. 20 and 22). The ends of the spindles are curved and project beyond the nuclear area even at metaphase (Pl. V, Fig. 22). Again by elongation of the spindle the daughter nuclei are separated so as to be arranged in four pairs, each pair consisting of two non-sister nuclei. The origin of the members of each pair is clearly shown by the position of the curved persistent end of the spindle, each daughter nucleus having one such end attached to its nuclear area giving it a beaked appearance (Text-fig. 6).

*Spore formation.* When spore formation begins the ascus contains eight nuclei corresponding to those which in most Ascomycetes form the nuclei of the eight spores. In *N. tetrasperma*, however, these eight nuclei are arranged in four pairs and a mass of cytoplasm rounds up about each of the pairs of non-sister nuclei. This results in four binucleate spores (Text-fig. 6). Binucleate spores are therefore the normal ones for *N. tetrasperma*. These increase in size, the nuclei lose their beaked appearance, and, as the spores attain their final dimensions, the nuclei undergo yet another division. At this division six chromosomes can be



counted at metaphase (Pl. V, Fig. 23). The nuclei are situated at the surface of the spore, and because of this the spindles are curved and it is not possible to obtain an exact count at anaphase. Some late anaphases, however, have been found in which four and occasionally five chromosomes are visible (Pl. V, Fig. 24). The combined evidence from the counts of the preceding division, and from metaphase and anaphase of the divisions in the spore, shows conclusively that the haploid number of chromosomes for *N. tetrasperma* is six. The mature spore in the four-spored asci is four-nucleate at maturity (Pl. V, Fig. 25) and becomes thick walled and black when ripe.

Sometimes, however, as has been previously stated, spore formation is irregular. When the cleavage of the protoplasm begins in the ascus, instead of a pair of nuclei being incorporated in each mass, either more than a pair, or only one of a pair, may be included in each spore. In all cases, as the spore matures, division of the nuclei occurs. It is the small spores initially uninucleate, though binucleate at maturity, which give rise to the heterothallic strains. Such a condition which results in more than four spores in the mature ascus, though exceptional, occurs in a considerable number of asci.

#### GERMINATION AND MYCELIAL FUSIONS.

The spores germinate only after being heated for about twenty minutes at a temperature between 70° and 90° C. (10). The mycelium which is produced consists of very long multinucleate cells. The transverse walls form characteristic collars round the cells in old hyphae (Pl. IV, Fig. 4).

Mycelial fusions between adjacent hyphae are found in great abundance in all the cultures, whether of single strains or of A and B growing together (Pl. IV, Fig. 4). Where inoculations of A and B are made at opposite sides of a Petri dish, each strain grows completely across the medium so that the two strains become intimately mixed. As a result of this the perithecia do not form in a line at the meeting of the two mycelia but are scattered over the whole surface. There seems to be, however, a greater concentration of perithecia on the B side of the cultures. Mycelial fusions evidently allow of a unity of action throughout the whole mycelium of any culture and are undoubtedly of great importance in the production of perithecia containing both A and B spores.

#### DISCUSSION.

The points of interest which arise from the study of this fungus are three in number. Firstly, the fact that only one reduction division and therefore only one nuclear fusion occurs in *Neurospora* has to be considered in connexion with other fungi where the details of the nuclear cycle are

known. Secondly, there is the influence of the nuclear difference in the two strains on perithecial formation and, thirdly, there is the bearing of the single reduction division and single fusion on the segregation of factors for heterothallism. These three points will form the basis of the following discussion.

(i) *The nuclear cycle in the Ascomycetes.* The cytological data for *N. tetrasperma* may be profitably recapitulated. The haploid number of chromosomes in *N. tetrasperma* is six. This is clear from the counts obtained from the divisions in the spore. The number of chromosomes at metaphase in the first division in the ascus is twelve, and at anaphase six pass to each pole, while at the second and third divisions there are six chromosomes at metaphase and six pass to each pole at anaphase. Thus there can be no doubt that there is only one reduction division in *N. tetrasperma*.

A review of the Ascomycetes which have been cytologically investigated, brings out the fact that they show a series of stages in the disappearance of syngamy. Normal fertilization with a fusion of sexual nuclei in the oogonium has been described for certain of the Erysiphales, notably for *Sphaerotheca Humuli* (25) (3), *Erysiphe Polygoni* (26), and *Phyllactinia Corylea* (28), and for some of the Discomycetes such as *Ascodesmis nigricans* (7), *Pyronema confluens* (27) (22), and *Ascobolus magnificus* (23). A fusion of oogonial nuclei in the absence of a functional antheridium has been recorded for *Lachnea stercorea* (17), and *Humaria granulata* (20) (21). A fusion of vegetative nuclei has been described for two fungi which do not possess morphologically defined sexual organs, i.e. *H. rutilans* (18) and, less convincingly, for *Helvella crispa* (6). In all these cases a second fusion in the ascus hook has been recorded, and in some of them (*P. confluens*, *H. granulata*, *A. magnificus*, and *H. rutilans*), the nuclear divisions in the ascus have been examined and reduction division followed by brachymeiosis has been observed.

In two cases, which are included in the above group, *P. confluens* (22) and *A. magnificus* (23) nuclei, probably those which failed to pair, are seen to remain in the oogonium and degenerate when the fusion nuclei have passed into the ascogenous hyphae. In *P. domesticum* (33), on the other hand, the unpaired nuclei do not degenerate, but pass with the fusion nuclei into the ascogenous hyphae so that the latter contain both haploid and diploid nuclei. After the fusion in the ascus hook, the young ascus may contain either a diploid or a tetraploid definitive nucleus. Reduction division takes place at the first division in all the asci, but brachymeiosis follows only where a tetraploid nucleus is concerned.

In *S. Humuli* (34) and *E. Polygoni* (9) among the Erysiphales, apogamous varieties have been described, where fertilization does not take place and the male nucleus degenerates in the antheridium. Among the

Discomycetes apogamy—that is complete absence of the first fusion—has been described for *P. confluens* var. *irigneum* (5), *A. citrinus* (32), *A. immersus* (31), and *Pustularia bolarioides* (1). In all these six cases a fusion in the ascus hook has been seen. The cytology of the ascus has not been investigated in *A. citrinus*, but *A. immersus*, and *P. confluens* var. *irigneum* are said to show the same number of chromosomes throughout the nuclear divisions in the ascus; in *P. bolarioides* only, has any clear account of reduction division been given. Presumably all these apogamous forms would on careful investigation show but a single reduction division.

The work on *N. tetrasperma* presents a clear instance of a single reduction division. This fact, coupled with the knowledge that *N. tetrasperma* possesses no antheridium and shows no evidence whatever of a first fusion, carries the suggestion of the occurrence of a simplified life-history into the realms of certainty. Such a fungus as *Neurospora*, which has only the fusion in the ascus hook, shows clearly the last stage in the loss of syngamy. In considering the correlation between the single fusion and single reduction division, it is not surprising to find that if one of the reduction divisions is rendered unnecessary it should be the first to take place which is retained, and the second shortened one which is lost.

The investigation of *Neurospora* also adds a member of the group of the Pyrenomycetes to the number of fungi of which the cytology of the ascus divisions is known. Very little critical work has been done on this group, and it is interesting to find the most simplified nuclear cycle appearing here. Cytological work on other Pyrenomycetes is desirable in order to ascertain whether any of them still retain both fusions and both reduction divisions in their life-history. From this review of the position it will be seen that the cases of Ascomycetous fungi, where only one fusion and one reduction division occur, can be regarded as modifications of the general plan and not fundamentally different from it.

(ii) *The relationship of perithecial formation to nuclear condition.* In considering the relationship of the two strains A and B in heterothallic fungi, the position in *Neurospora* is instructive. The two strains both bear identical archicarps, and therefore they are like those of *H. granulata* (22), morphologically indistinguishable and so truly heterothallic in Blakeslee's original meaning of the term (4). As already mentioned, the strains A and B are obtained from spores which are initially uninucleate and when the two strains are grown separately no perithecia are formed. Presumably, a mixing of cell contents of the two strains is necessary for the development of asci from the archicarp. This mixing is initiated by means of mycelial fusions. The nuclei from both the strains must be present in the archicarp if development is to continue. They must pass at random into the ascogenous hyphae and, where two nuclei from the different strains are adjacent, initiate ascus hooks. Fusion occurs here, giving rise to diploid

nuclei carrying both the A and the B characters, and segregation follows immediately at reduction division. In 1928 Dodge (12) succeeded in obtaining fertile hybrids between *N. sitophila* and *N. tetrasperma* by

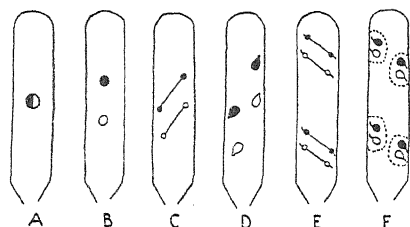


DIAGRAM 1. Segregation in the first division in the ascus; A-F show the stages in the formation of four homothallic spores.

growing one heterothallic strain of the former in contact with the reciprocal heterothallic strain of the latter. From these hybrids, by back crosses, he re-obtained the parental types. This experiment showed conclusively, that at some point in the life-history of these fungi, a fusion of unlike nuclei must take place. The suggestion that both types of nuclei are present in the ascogenous hyphae and that they fuse in the ascus hook provides a logical explanation of Dodge's results.

The homothallic cultures are obtained from the normal (initially binucleate) spores. These spores on germination produce mycelia which are already provided with both kinds of nuclei. Functional archicarpes are produced and the formation of a diploid nucleus carrying both characters is again the result of fusions in the ascus hooks. Segregation of the two characters will again follow at reduction division.

Thus it may be seen that *N. tetrasperma* is only homothallic because of the inclusion of two nuclei of different character in each young normal spore. It is only when these two nuclei are separated that their difference from one another—due to segregation of characters at reduction division—is manifest. In other words, the homothallic nature of *N. tetrasperma* is a secondary condition and different from the primary homothallism of eight-spored fungi such as *P. confluens*. It is probable that the four-spored homothallic condition of *N. tetrasperma* is a development from an earlier state where the formation of eight heterothallic spores was the normal condition, a state of affairs still shown by *Neurosperma sitophila* and *N. crassa* (10).

(iii) *Segregation in the ascus.* With regard to the question of the segregation of characters for heterothallism it is obvious that segregation must, in the absence of crossing-over, take place at the first division in the ascus, since this is the reduction division (Diagram 1). The nuclei, after each division, are rearranged (p. 219), and this arrangement is such that each

young binucleate spore possesses two unlike (i.e. an A and a B) nuclei, each young uninucleate small spore either an A or a B nucleus only.

In the event of crossing-over taking place during reduction division,

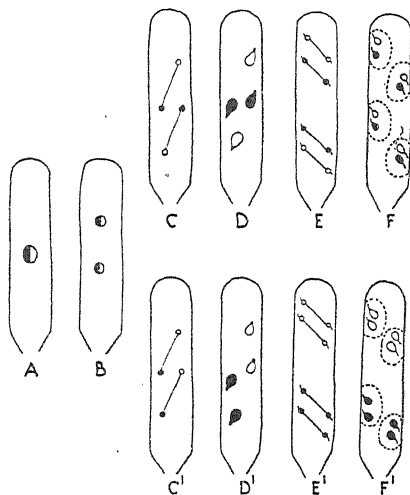


DIAGRAM 2. Segregation in the second division in the event of crossing over; A, B, and C-F show the formation of four homothallic spores if segregation is as in C; A, B, and C'-F' show the formation of four heterothallic spores if segregation is as in C'.

segregation would be delayed until the second division, and in these cases a certain proportion of normal spores in four-spored asci would contain when young either two A or two B nuclei. These spores would, on germination, give rise to heterothallic not homothallic strains (Diagram 2). Up to the present no case of crossing-over has been recorded by Dodge in his genetical work. Calculation makes it clear that it would be encountered only infrequently, and single-spore cultures of initially binucleate spores from four-spored asci have not been made in sufficient numbers for the phenomenon, if it occurs, to have manifested itself.

#### SUMMARY.

1. *N. tetrasperma* (Dodge) is a normally homothallic fungus from which heterothallic strains have been obtained.
2. The heterothallic strains both bear archicarps of exactly similar appearance to those which form in homothallic cultures. Antheridia are not present in any of the cultures.
3. The cells of the mycelium and archicarp are multinucleate throughout development.
4. The development of the archicarps is arrested in single strain cultures after a sheath has been formed. These abortive archicarps form sclerotia and act as accessory reproductive bodies.

5. No nuclear fusion has been found in the early stages of development of the perithecium. The fusion in the young ascus seems to be the first and only one.

6. The definitive nucleus of the ascus is diploid with twelve chromosomes; reduction division takes place at the first division in the ascus.

7. The subsequent divisions are all equational ones and the haploid number of chromosomes is six.

8. Two non-sister nuclei become included in each young normal spore. Occasionally these two nuclei are separated and each is included in a small spore. The latter, though only occasionally found in *N. tetrasperma*, correspond to the spores of *N. sitophila*, eight of which are developed in each ascus.

9. A mitotic division takes place in the nuclei of all the spores, so that the normal spore is four nucleate and the small spore binucleate at maturity.

10. Numerous mycelial fusions occur in all the cultures.

11. The nuclear cycle of *N. tetrasperma* by comparison with that of the other Ascomycetes is shown to be the last stage in the disappearance of syngamy.

12. The relationship of the nuclei of the spores to the production of the perithecia is discussed, and the four-spored condition of *N. tetrasperma* is concluded to be derived from an earlier eight-spored form.

13. Segregation is shown to occur in the first division in the ascus. The possibility of crossing over with its effect on the position of segregation and the nuclear content of the spores is considered.

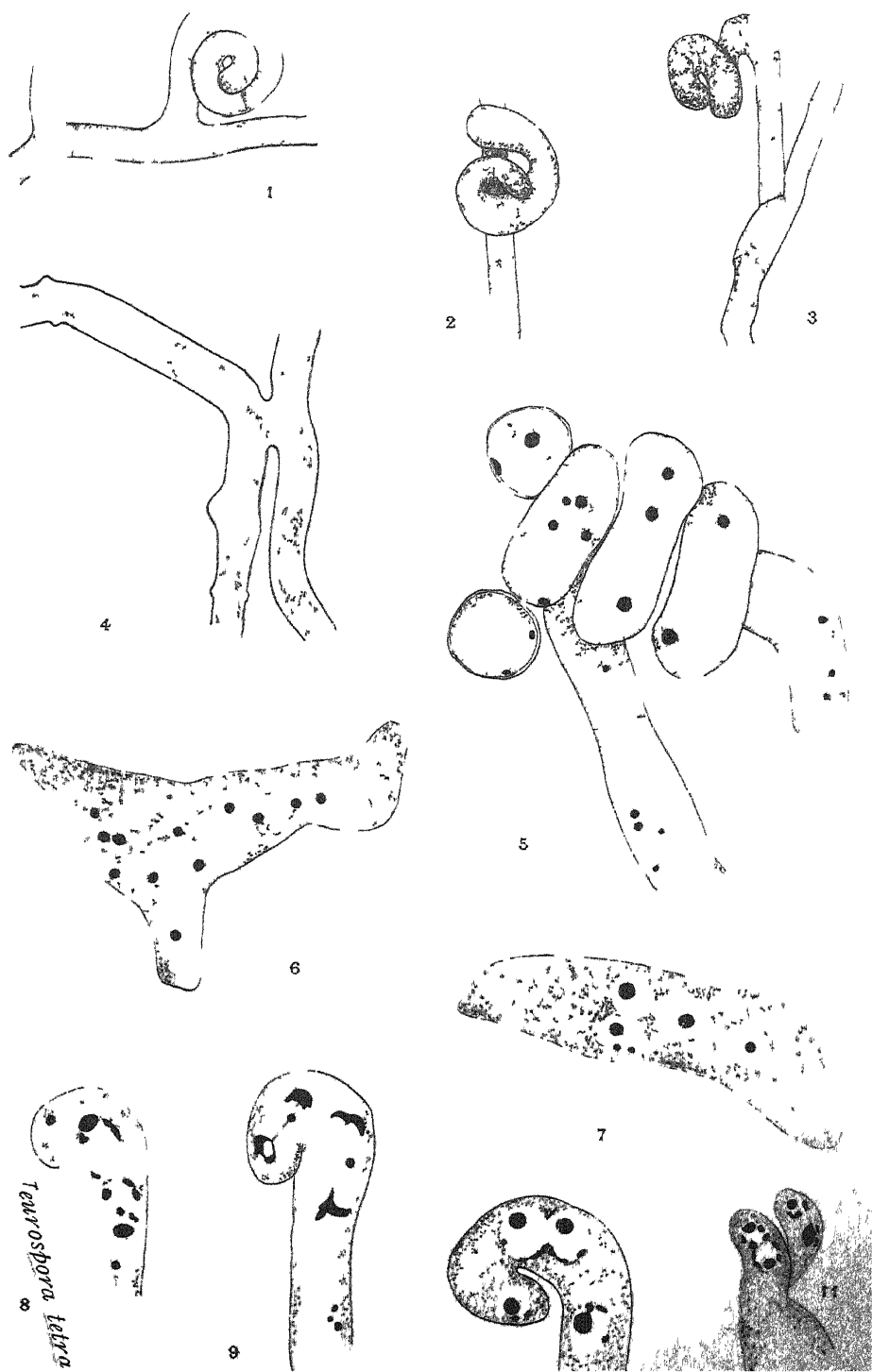
In conclusion, I wish to express my thanks again to Dr. Dodge for his gift of the cultures; to Professor Dame Helen Gwynne-Vaughan and to Professor Lang for help and criticism during the course of the work.

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December 1932.

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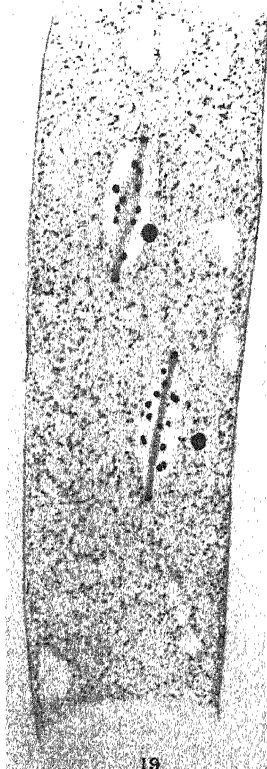
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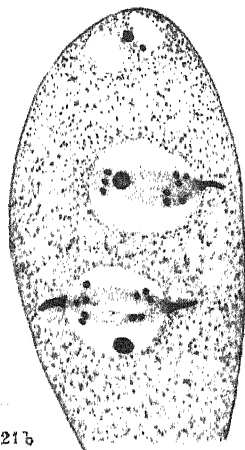
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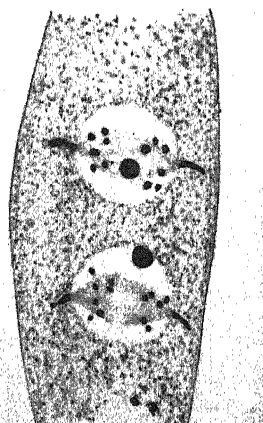
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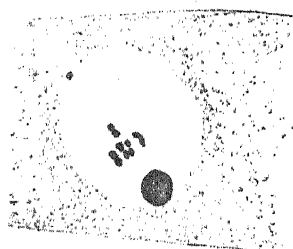
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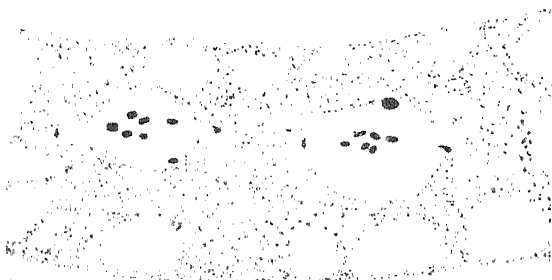
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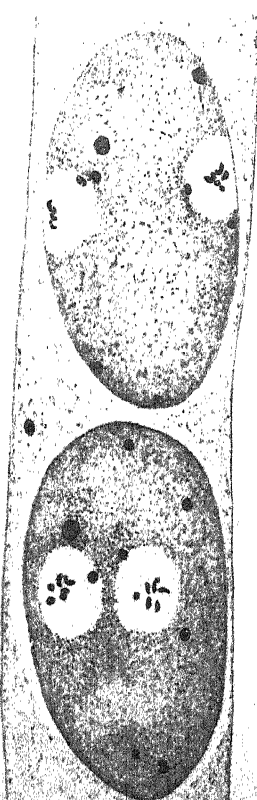
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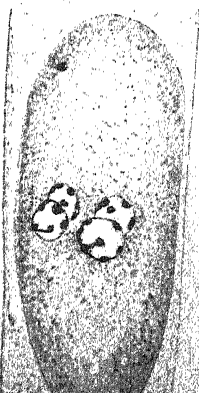
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# The Salt Marshes of the Dovey Estuary.

## IV. The Rates of Vertical Accretion, Horizontal Extension and Scarp Erosion.<sup>1</sup>

BY

F. J. RICHARDS.

With ten Figures in the Text.

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### I. INTRODUCTION.

IN 1916 and 1917 appeared an account by Yapp, Johns, and Jones (6 and 7) of the salt marshes of the Dovey Estuary, special attention being given to phytogeological problems, i.e. 'the effect of salt marsh plants in modifying and controlling geological processes'. Five vegetation zones were distinguished, each occupying a comparatively small vertical range. As the marsh is built up, the vegetation normally passes through the following well-defined associations : (1) *Salicornietum europaeae*, (2) *Glycerietum*

<sup>1</sup> The present communication is a further account of researches into the processes of the building and subsequent history of a salt marsh, conducted until his death by Professor R. H. Yapp. Parts I, II, and III of this series appeared in the Journal of Ecology, vols. iv, v, and x respectively (1916, 1917, and 1922).

*maritimae*, (3) *Armerietum maritimae*, (4) *Festucetum rubrae*, and (5) *Juncetum maritimi*. The authors successfully elucidated the process of the primary building of the marsh together with secondary changes in surface relief, including marginal erosion, formation of secondary marsh, and modifications of channels and pans. These processes were studied by a comparison of the different stages found on the marsh, and therefore 'afforded little indication of the rate at which the various progressive and retrogressive changes proceed'.

This account was followed by a further short paper in 1922 (5), dealing with the rate of change of pan systems as observed over a period of seven years.

In the summer of that year Professor Yapp conducted a volunteer party<sup>1</sup> to the salt marsh, in order to carry out the preliminary work for a more detailed study of the rates of the various processes which normally take place. Certain areas which were then surveyed were re-surveyed in 1925, and data concerning the rate of vertical accretion were collected in 1926. On the lamented death of Professor Yapp the investigation passed to the hands of the author, who visited the marshes again in April 1930, and collected the final data on which this paper is based. The design of the experiments is thus exclusively Professor Yapp's, but for the working out of the results and methods of presentation the author is alone responsible.

The main part of the work deals with the rate of vertical accretion of the sward associations of the marsh, but data are also presented concerning the rate of colonization of bare silt by *Glyceria*, growth of the young marsh, rate of erosion of the river front, and of the formation of secondary marsh.

## 2. RATE OF VERTICAL ACCRETION.

### (a) *General.*

The rate of upward growth of the marsh was studied by spreading on the soil surface a paste, made by mixing certain coloured materials with sea water, and after the lapse of a few years determining the depth of accumulated silt above the substances. The actual materials used were puce-coloured and light red Alum Bay sands, brick dust and coal dust. These were laid down during the first week of July 1922 in four areas of the saltings; strips about 8 in. across running approximately north and south were laid over the whole width of the marsh, from the limit of vegetation at the river side to the *Juncetum* at the landward (southern)

<sup>1</sup> This consisted of Dr. J. S. Bayliss Elliott, Miss U. C. Slane, Miss E. Tacon, Miss F. G. Wood, Messrs. A. J. Barnard, W. J. Millard, W. A. S. Perring, and F. J. Richards. Assistance at later stages in the work was given by Miss E. Mason, Messrs. W. Leach and E. W. Rogers, and Dr. J. H. Salter.

extremity, a distance of approximately 100 yards. Two of these strips were laid at the Ynyslas end, towards the seaward limit of the marshes in the estuary (near A on the map in (7), Fig. 1), and two more at the Glandovey end, some three miles to the eastward, and near the head of the estuary (at C on the map). These strips will be referred to in the sequel as Lines I-IV, Line I being the most westerly, at the Ynyslas end, and Line IV the easternmost, at the Glandovey end.

In addition a fifth line running east and west was laid down in May, 1925, on the new and rapidly growing marsh to the east, and near the river end of the great breakwater (B on the map) in the Glandovey area. Each line was laid uniformly over the sward, channels, and bare pans as these occurred along its course. It was impossible to avoid contaminating the leaves of the vegetation with the pastes, but the first tide or so was sufficient to wash them clean, and the plants were entirely uninjured. The materials were gradually buried by the deposits accumulating on the marsh surface. Where the vegetation was closed the lines persisted, but in very open *Glycerietum* the materials were frequently completely dissipated before burial, while on bare silt it was rarely that any trace of them could be detected later. When the lines were subsequently dug up it was found that both the red and the black colours usually stood out conspicuously against the grey of the accumulated silt; on the whole the black was probably more striking, except in certain localities (usually low-lying or waterlogged as at the edges of pans) where the silt itself was partially discoloured and blackish. The coloured layer showed a small but measurable thickness in the deposits, and the amount of accretion has been measured from the soil surface to the top of the line, which in nearly all cases presented a sharp edge.

During the first half of September 1926, the lines were dug up at a number of representative points spaced at intervals along them of roughly 5-10 ft., according to local topography. In every case the distance of the point from the estuary limit of the marsh was recorded, together with the nature of the vegetation, the amount which the soil surface had risen since the line was laid down, and so far as possible any peculiarities of local conditions. In addition, levels were taken along Lines III and V at the observed points, so that their heights above the general silt level were known.

At this stage the investigation passed into the hands of the author. An examination of the data collected showed that the rate of accretion was so dependent on the height of the point that it was impossible to trace clearly its connexion with other factors while this was unknown. For this reason Lines I, II, and IV were again dug up in the first half of April 1930, and levels taken at the observed points. In the sequel the data collected in 1926 are used for Lines III and V, while those of 1930 are used in the case of Lines I, II, and IV. Since Line V gives

information concerning a peculiar set of conditions brought about by the presence of a breakwater, to which indeed this part of the marsh owes its existence, the results from it will be considered only after the data relating to the general history of marsh developed under normal conditions have been discussed.

The data given by an experiment of this kind are exceedingly difficult to interpret by inspection, owing to the fact that the observed accretion rate is dependent on several factors, which are themselves varying more or less independently of one another. The number of concrete conclusions which may be drawn from them in this manner is clearly limited, and such as can be drawn are subject in large measure to personal bias. For this reason statistical methods have been freely used; by means of them personal bias is eliminated, all the relevant evidence is taken into account, the most probable relationships between the several factors are clearly revealed, and the probability of these relationships being real or spurious is given in the form of odds. Modern statistical methods present the most reliable and efficient analysis of such a mass of data, and their adoption wherever possible cannot be too strongly urged.

#### (b) *Statistical Methods.*

The methods used in the statistical study of the results are those of correlation and regression, which are dealt with in detail by Fisher (3), and by Ezekiel (2). Suffice it to say here that from an examination of two variable quantities, it is possible to determine a numerical value, the 'correlation coefficient', from whose magnitude the probability of a real interdependence existing between the quantities may be determined. The correlation coefficient, denoted by  $r_{xy}$ , where  $x$  and  $y$  are the variables or 'variates', may assume any value between  $+1$  and  $-1$ , these two extremes being found if the correlation is perfect; values nearer zero indicate that the correlation is not so marked. Positive values show that departures from the mean value of one factor are in general accompanied by departures in the same direction from the mean value of the other factor, and negative values that such deviations are mainly in opposite directions.

It is often found that a high and statistically significant correlation coefficient may be obtained from two variates, when in reality one is not directly dependent on the other, but both vary with some third quantity. When this is the case it is possible to disentangle the complex relationships, and to eliminate from the total correlation between  $x$  and  $y$ , that part which is due to the third variate,  $z$ . The result is expressed as a 'partial correlation coefficient', denoted in this case by  $r_{xy.z}$ . This coefficient also may have values ranging between  $+1$  and  $-1$ .

When it is desired to assess the extent to which a varying quantity  $x$  is determined by two or more other variates,  $y$ ,  $z$ , &c. considered together,

a third measure of correlation, the 'multiple correlation coefficient', may be used. This is denoted by  $R_{x.yz}$ , and may assume values between +1 and 0, the former being obtained if variations in  $x$ , the 'dependent' variate, are completely accounted for by the corresponding variations in  $y$ ,  $z$ , &c., the 'independent' variates; and the latter if the values assumed by  $x$  are completely independent of the corresponding values of  $y$ ,  $z$ , &c. These three correlation coefficients, when the size of the sample from which they are calculated is taken into account, may be expressed in terms of the probability of the relationship under examination being a real one, and not due to chance causes. If the probability exceeds 20:1 (the '5 per cent. point') the relationship is usually accepted as being 'significant'; a much more stringent test is the 1 per cent. point, this being the value which the coefficient must attain if it is to have a probability of significance of 100:1.

A relationship such as is studied by means of the multiple correlation coefficient may be expressed in the form of a 'regression equation', which gives as good a measure as possible of the manner in which change of the dependent variate is determined by changes of the various independent variates, e.g.

$$x = a.y + b.z + c,$$

where  $a$ ,  $b$ , and  $c$  are constants. Such an equation has the probability of significance which is given by the multiple correlation coefficient  $R_{x.yz}$ . Where the relationship between  $x$  and any of the independent variates cannot be represented adequately by a straight line, terms involving some function (as the square or reciprocal) of the given variates may be introduced into the right-hand side of the equation. In the present work logarithms of the variates have been introduced, the equation taking the form:

$$x = a.y + b.\log y + c.z + d.\log z + e,$$

where  $d$  and  $e$  are additional constants.

### (c) *Results.*

Seeing that the method of treatment of the data automatically extracts from them all the relevant information, there is no need to present them *in extenso*, and the analysis may be proceeded with immediately. The lines will be dealt with individually, seeing that conditions are not uniform over the whole of the marsh. The variates analysed are (1) amount of vertical accretion in a given time (referred to in formulae as ' $a$ '), (2) height of the marsh surface above the level of the bare silt at the river extremity of the line (' $h$ '), and (3) distance along the line from the erosion scarp which is found almost at the extreme river end of the line (' $d$ '). Below this scarp a small amount of secondary marsh occurs, but since conditions

there differ from those on the primary marsh, the few observations beyond the scarp have been neglected for the purpose of the following correlations.

### LINE I.

Total length approximately 400 ft. ; 1930 data. The following table gives the mean values for accretion and land height, at the observed points :

Association.	Accretion (cm. in 100 lunar months).	Land height (feet).	Number of observations.
<i>Glycerietum</i>	6.61	1.65	8
Trans. <i>Glyc.</i> → <i>Arm.</i>	5.53	1.53	7
<i>Armerietum</i>	3.53	2.13	30
Trans. <i>Arm.</i> → <i>Fest.</i>	2.00	2.85	1
<i>Festucetum</i>	1.75	3.24	4
<i>Juncetum</i>	2.03	2.69	3
Total			53

It is seen that in this area, as we pass through the successive vegetation zones, the height of the marsh rises fairly uniformly, while the rate of accretion drops rapidly. The following are the total and partial correlation coefficients obtained between the three variables concerned :

$$\begin{array}{ll} r_{ah} = -0.8115 & r_{ah.d} = -0.782 \\ r_{ad} = -0.5134 & r_{ad.h} = -0.401 \end{array} \quad \left. \vphantom{\begin{array}{l} r_{ah} \\ r_{ad} \end{array}} \right\} 1\% = 0.354.$$

$$r_{dh} = +0.3635$$

There are close negative total correlations between accretion and land height on the one hand and accretion and scarp distance on the other, and when these are expressed as partial correlation coefficients they are still found to be clearly significant. The multiple correlation coefficient, showing the degree of dependence of accretion rate on the other two variates, is  $R_{a.hd} = 0.845$  (1 per cent. = 0.41). Thus by treating this line as a whole and assuming a linear relationship between accretion rate and each of the independent variates, over 71 per cent. (i.e.  $0.845^2$ ) of the total variance in the observed accretion rates is accounted for, leaving less than 29 per cent. still to be explained. The corresponding regression equation is :

$$\text{Accretion}^1 = -2.3673 (\text{Land Height}) - 0.004275 (\text{Scarp Distance}) + 9.7559.$$

By subdividing these observations into vegetation groups rather more information may be gained. The results may be summarized as follows :

#### *Glycerietum.*

$$\begin{array}{ll} r_{ah} = -0.8039 & r_{ah.d} = -0.774 \\ r_{ad} = -0.6391 & r_{ad.h} = -0.574 \end{array} \quad \left. \vphantom{\begin{array}{l} r_{ah} \\ r_{ad} \end{array}} \right\} 5\% = 0.755.$$

$$r_{dh} = +0.4074$$

$$R_{a.hd} = 0.873 \quad (5\% = 0.836; 1\% = 0.917).$$

<sup>1</sup> In this, as in all subsequent equations, it should be noted that land height and scarp distance are expressed in feet while amount of accretion is measured in centimetres. The time period corresponding to this accretion is 100 lunar months.



Transitional: *Glycerietum* to *Armerietum*.

$$\begin{array}{l} r_{ah} = -0.2749 \\ r_{ad} = -0.5656 \\ r_{dh} = +0.8591 \end{array} \quad \begin{array}{l} r_{ah,d} = +0.500 \\ r_{ad,h} = -0.670 \end{array} \left. \vphantom{\begin{array}{l} r_{ah} \\ r_{ad} \\ r_{dh} \end{array}} \right\} 5\% = 0.811.$$

$$R_{a,h,d} = 0.700 (5\% = 0.881).$$

*Armerietum*.

$$\begin{array}{l} r_{ah} = -0.7465 \\ r_{ad} = -0.4560 \\ r_{dh} = +0.2548 \end{array} \quad \begin{array}{l} r_{ah,d} = -0.732 \\ r_{ad,h} = -0.413 \end{array} \left. \vphantom{\begin{array}{l} r_{ah} \\ r_{ad} \\ r_{dh} \end{array}} \right\} \begin{array}{l} 5\% = 0.368; \\ 1\% = 0.472. \end{array}$$

$$R_{a,h,d} = 0.795 (1\% = 0.53).$$

$$\text{Accretion} = -2.2575 \text{ (Land height)} - 0.0030805 \text{ (Scarp distance)} + 8.9593.$$

No other vegetation zone has a sufficient number of observed points to warrant further investigation. Concerning the above correlations it may be pointed out that the partial coefficient between accretion and scarp distance in the *Glycerietum*, although greater than in the line treated as a whole, is not significant, owing to the small number of observed points. In the transitional zone it is interesting to note that the correlation between accretion and height, though not significant, is yet marked and *positive*; this will be referred to later. Finally, the very close similarity between the regression equation derived from the thirty *Armerietum* points, and that derived from the total fifty-three points on the line, should be noted. These results will be discussed when the general results obtained from the other lines have been presented.

LINE II.

Total length approximately 280 ft.; 1930 data.

Association.	Accretion (cm. in 100 lunar months).	Land height (feet).	Number of observations.
<i>Glycerietum</i>	3.50	1.25	1
Trans. <i>Glyc.</i> → <i>Arm.</i>	6.29	1.55	13
<i>Armerietum</i>	3.70	1.42	14
Trans. <i>Arm.</i> → <i>Fest.</i>	3.32	1.87	6
<i>Festucetum</i>	3.31	2.23	9
<i>Juncetum</i>	1.20	2.96	1
Total			44

The accretion rates call for some comment, seeing that the general run is different from that on Line I, there being an exaggerated maximum value in the transitional vegetation between the *Glycerietum* and *Armerietum*. In this figure are included three very high values (mean = 12.2 cm.), these being observations within a few feet of the scarp, where the accretion rate, as will appear later, is much greater than elsewhere on the marsh. But even if these three points are omitted the mean of the remaining ten is still high, namely 4.52 cm.

The correlation coefficients for the total line, and for the various vegetation sub-divisions are as follows:

## Total Line.

$$\begin{array}{ll} r_{ah} = -0.3122 & r_{ah.d} = -0.543 \\ r_{ad} = -0.3878 & r_{ad.h} = -0.580 \end{array} \quad 1\% = 0.39.$$

$$R_{a.hd} = 0.633 \quad (1\% = 0.454).$$

Transitional: *Glycerietum* to *Armerietum*.

$$\begin{array}{ll} r_{ah} = -0.0505 & r_{ah.d} = +0.399 \\ r_{ad} = -0.9705 & r_{ad.h} = -0.975 \end{array} \quad \begin{array}{l} 5\% = 0.576; \\ 1\% = 0.708. \end{array}$$

$$r_{dh} = +0.1500$$

$$R_{a.hd} = 0.975 \quad (1\% = 0.776).$$

$$\text{Accretion} = +1.7691 \text{ (Land height)} - 0.032616 \text{ (Scarp distance)} + 9.2230.$$

*Armerietum*.

$$\begin{array}{ll} r_{ah} = -0.5758 & r_{ah.d} = -0.691 \\ r_{ad} = +0.0218 & r_{ad.h} = -0.467 \end{array} \quad \begin{array}{l} 5\% = 0.553; \\ 1\% = 0.684. \end{array}$$

$$r_{dh} = -0.5782$$

$$R_{a.hd} = 0.691 \quad (5\% = 0.648; 1\% = 0.753).$$

$$\text{Accretion} = -1.8291 \text{ (Land height)} - 0.010375 \text{ (Scarp distance)} + 7.8003.$$

Transitional: *Armerietum* to *Festucetum*, with *Festucetum*.

$$\begin{array}{ll} r_{ah} = -0.8246 & r_{ah.d} = -0.274 \\ r_{ad} = +0.8161 & r_{ad.h} = +0.187 \end{array} \quad 5\% = 0.532.$$

$$r_{dh} = -0.9494$$

$$R_{a.hd} = 0.831 \quad (1\% = 0.732).$$

The transitional vegetation and the pure *Festucetum* have here been treated together, since an examination of the graphs of accretion rate and land contour shows that no difference in behaviour exists between them.

## LINE III.

Total length approximately 250 feet; 1926 data.

Association.	Accretion (cm. in 54 lunar months).	Land height (feet).	Number of observations.
<i>Glycerietum</i>	3.67	1.15	6
Trans. <i>Glyc.</i> → <i>Arm.</i>	6.93	1.64	2
<i>Armerietum</i>	4.09	1.67	15
<i>Festucetum</i>	3.38	2.31	6
Trans. <i>Fest.</i> → <i>Junc.</i>	4.60	2.33	1
<i>Juncetum</i>	2.25	3.00	1
Total			31

From these figures it appears that in this area also accretion rate passes through a maximum as the land rises. The correlation coefficients obtained along this line are as follows:

## Total Line.

$$\begin{array}{ll} r_{ah} = -0.0681 & r_{ah.d} = -0.152 \\ r_{ad} = -0.1891 & r_{ad.h} = -0.393 \end{array} \quad 5\% = 0.362.$$

$$r_{dh} = -0.3724$$

*Glycerietum.*

$$\begin{array}{ll} r_{ah} = +0.7255 & r_{ah,d} = +0.570 \\ r_{ad} = -0.6872 & r_{ad,h} = -0.498 \end{array} \left. \vphantom{\begin{array}{l} r_{ah} \\ r_{ad} \end{array}} \right\} 5\% = 0.878.$$

$$r_{dh} = -0.5540$$

*Armerietum.*

$$\begin{array}{ll} r_{ah} = -0.2260 & r_{ah,d} = -0.634 \\ r_{ad} = -0.7391 & r_{ad,h} = -0.845 \end{array} \left. \vphantom{\begin{array}{l} r_{ah} \\ r_{ad} \end{array}} \right\} \begin{array}{l} 5\% = 0.532; \\ 1\% = 0.661. \end{array}$$

$$r_{dh} = -0.2531$$

$$R_{a,h,d} = 0.854 (1\% = 0.732).$$

$$\text{Accretion}^1 = -1.2213 (\text{Land height}) - 0.010,317 (\text{Scarp distance}) + 7.1389.$$

*Festucetum.*

$$\begin{array}{ll} r_{ah} = +0.6855 & r_{ah,d} = -0.016 \\ r_{ad} = -0.7376 & r_{ad,h} = -0.374 \end{array} \left. \vphantom{\begin{array}{l} r_{ah} \\ r_{ad} \end{array}} \right\} 5\% = 0.878.$$

$$r_{dh} = -0.9347$$

$$R_{a,h,d} = 0.738 (5\% = 0.930).$$

LINE IV.

Total length approximately 230 feet; 1930 data. The correlations are everywhere low.

Association.	Accretion (cm. in 100 lunar months).	Land height (feet).	Number of observations.
Trans. <i>Glyc.</i> → <i>Arm.</i>	6.54	1.51	10
<i>Armerietum.</i>	6.12	1.82	28
Trans. <i>Arm.</i> → <i>Fest.</i>	5.10	2.03	3
<i>Juncetum</i>	5.10	2.09	1
Total			42

Total Line.

$$\begin{array}{ll} r_{ah} = -0.0558 & r_{ah,d} = -0.074 \\ r_{ad} = +0.2068 & r_{ad,h} = +0.212 \end{array} \left. \vphantom{\begin{array}{l} r_{ah} \\ r_{ad} \end{array}} \right\} 5\% = 0.304.$$

$$r_{dh} = +0.0793$$

Transitional: *Glycerietum* to *Armerietum.*

$$\begin{array}{ll} r_{ah} = -0.1743 & r_{ah,d} = -0.192 \\ r_{ad} = +0.0926 & r_{ad,h} = -0.123 \end{array} \left. \vphantom{\begin{array}{l} r_{ah} \\ r_{ad} \end{array}} \right\} 5\% = 0.666.$$

$$r_{dh} = -0.8716$$

*Armerietum.*

$$\begin{array}{ll} r_{ah} = +0.1488 & r_{ah,d} = +0.252 \\ r_{ad} = +0.4972 & r_{ad,h} = +0.528 \end{array} \left. \vphantom{\begin{array}{l} r_{ah} \\ r_{ad} \end{array}} \right\} \begin{array}{l} 5\% = 0.381; \\ 1\% = 0.487. \end{array}$$

$$r_{dh} = -0.1365$$

(d) Discussion.

The results which have been presented above will now be reviewed. In the first place it may be mentioned that while in some older areas of the marsh (Line I) accretion rate is apparently almost completely determined by situation on the marsh, and may be expressed in terms of land height

<sup>1</sup> On this line the time period corresponding to the amount of accretion given by the regression equation is 54 lunar months.

and scarp distance, in others (Lines II and III) no such satisfactory general relationship is found, and in order to determine the accretion rate of any point with approximate accuracy account must be taken, not only of situation, but also of the type of vegetation at that point; in yet other areas (Line IV) accretion rate is not even largely determined by situation and vegetation, and comparatively low correlations are found everywhere. It will be convenient to discuss the results from the different vegetation zones separately, in the following order: (1) *Armerietum*, (2) vegetation preceding *Armerietum*, (3) vegetation succeeding *Armerietum*.

(1) *Armerietum*.

On three lines highly significant negative correlations are found between accretion rate and height, as might be expected from the obvious fact that the higher the point is situated the fewer will be the tides reaching it and the smaller the amount of material available for upward growth. On Line IV the small positive correlation is negligible. Except on Line IV negative correlations are obtained between accretion rate and scarp distance, and on Lines I and III these are highly significant. An examination of the data indicates that the high positive correlation obtained on Line IV must be due to five abnormally high readings at the extreme landward end of the marsh. If we neglect this area, represented by the last 30 feet in a line 230 feet long, the positive correlations with both distance and height are replaced by negative though not significant values:

$$\begin{array}{ll} r_{ah} = -0.1845 & r_{ah,d} = -0.332 \\ r_{ad} = -0.0244 & r_{ad,h} = -0.282 \end{array} \left. \vphantom{\begin{array}{l} r_{ah} \\ r_{ad} \end{array}} \right\} 5\% = 0.482. \\ r_{dh} = -0.7893$$

The most that can be said of Line IV then is that accretion rate is far from being completely determined by a consideration of height and position on the marsh alone, but that 'chance' causes not included in these two factors assume large proportions; so large indeed that at the landward end, where, owing to the relatively high land together with the great distance from the river scarp, low accretion rates might normally be expected, the highest rates in the *Armerietum* have actually been recorded. One possible cause for the irregularity here is that the line is situated parallel to a breakwater, being distant approximately 40 yards from it; this must introduce unusual features into the tidal currents of the area.

The regression equation for the *Armerietum* in the area of Line I at the Ynyslas end of the marsh, though giving generally a very good fit between calculated and observed rates of accretion, is chiefly at fault at the scarp end of the line. This error can be practically eliminated by introducing some function of the variable concerned, so that the regression is not confined to a straight line but may assume a curved form. It is found that by introducing the logarithm of scarp distance the improvement in fit is large

and very highly significant. To determine whether this is the case or not Fisher's 'z' test may be used. The result in the present instance is given in the following table:

Variance due to	Degrees of freedom.	Sum of squares.	Mean square.	Z.	
Linear regression	2	30.4890			
Log. contribution	1	11.0888	11.0888	1.888	(1 % = 1.022).
Remainder	26	6.6046	0.25402		
Total	29	48.1824			

The multiple correlation coefficient corresponding to this regression can be determined from the figures in the 'Sum of Squares' column:

$$R = \sqrt{\frac{30.4890 + 11.0888}{48.1824}} = 0.929 \text{ (1 \% = 0.591).}$$

The regression equation is:

$$\text{Accretion} = 1.30401 \text{ (Height)} + 0.00827615 \text{ (Distance)} \\ - 3.70930 \text{ (Log}_{10} \text{ Distance)} + 12.6838,$$

and accounts for 86.3 per cent. of the total variance of the observed accretion rates. It must not be assumed from this equation, of course, that the relationship between accretion rate and scarp distance is necessarily of a logarithmic nature; in fact it is possible that had some other function been introduced in place of the logarithm a yet closer agreement might have been obtained. Logarithms are used merely in order to provide a curved regression from which it is possible to form a general idea of the shape of the curve relating accretion to distance.

In Fig. 1 are shown (1) the contour of the observed *Armeria* points along Line I, (2) the observed accretion rates at these points, and (3) the accretion values calculated from the above equation, for comparison with the actual rates. The general agreement is very striking.

It is of course possible to introduce some function of land height into the equation in order to obtain a curved regression on this variable also. If the logarithm is again used the equation becomes:

$$\text{Accretion} = -3.4713 \text{ (Height)} + 10.444 \text{ (Log}_{10} \text{ Height)} + 0.0077706 \\ \text{(Distance)} - 3.6555 \text{ (Log}_{10} \text{ Distance)} + 13.9292.$$

The improvement in fit obtained by this introduction is very slight, and is probably due entirely to chance, as the 'z' test shows. In fact, the regression of accretion on land height derived from this equation, over the actual range of observed height, departs very slightly indeed from rectilinearity.

If it is desired to determine the shape of the regression of accretion on, say, scarp distance, this may be done from the preceding equation by putting land height at a constant value, and solving the resulting equation

for accretion at a number of representative distances. Or a solid model may be constructed from the equation, giving the calculated rate at all combinations of height and distance. Since such a model cannot be

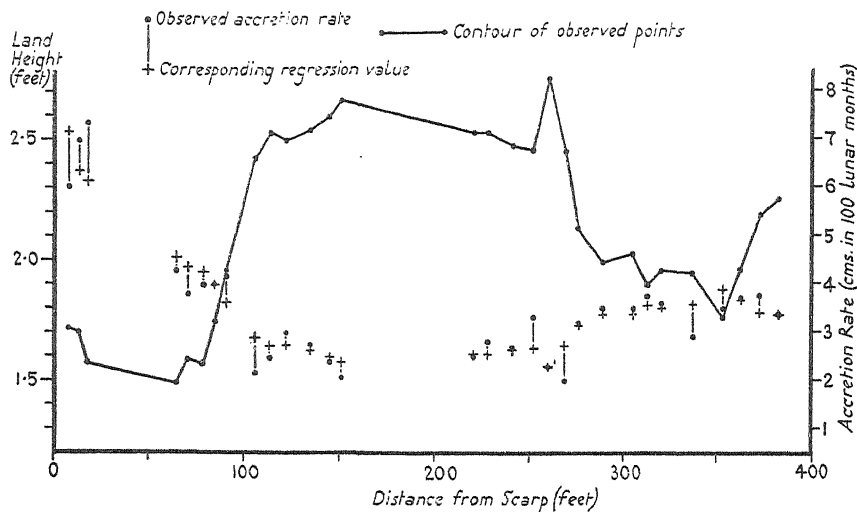


FIG. 1. Diagram showing, for the *Armerietum* of Line I, (1) contour of the observed points, (2) amounts of accretion observed at these points (cm. in 100 lunar months), and (3) accretion values calculated from the regression equation.

reproduced satisfactorily on a two-dimensional medium, a diagram is presented (Fig. 2) giving contours of the accretion rate surface as they would occur on the solid model constructed from the last equation, covering the observed ranges of height and distance in the *Armerietum*.

It may be of some interest to point out the method used in the construction of this figure. If in the right hand side of the equation we put:

$$3.4713 (\text{Height}) - 10.444 (\text{Log}_{10} \text{Height}) = 0.0077706 (\text{Distance}) \\ - 3.6555 (\text{Log}_{10} \text{Distance}) \dots (a)$$

it follows that the accretion value must be 13.9292. In other words, all the possible combinations of height and distance which satisfy equation (a) constitute the contour line of 13.9292. By adding the appropriate constant to the right-hand side of this equation, and solving for height at a number of distances, any desired contour may be thus mapped out. The equation (a) is best solved graphically, by plotting the left-hand side ('y') against land height over the required range; the height corresponding to any given distance can then be read off directly from the graph by determining from the right-hand side the value of 'y' corresponding to that distance.

In such a model as is represented by this figure, all sections parallel to either axis cut the accretion surface in parallel curved lines which are at different levels in the accretion dimension, and these sections are the

regressions of accretion on the variables considered. It should be mentioned that had the regression equation involving the linear term only of height been used a very similar diagram would have been obtained, with the

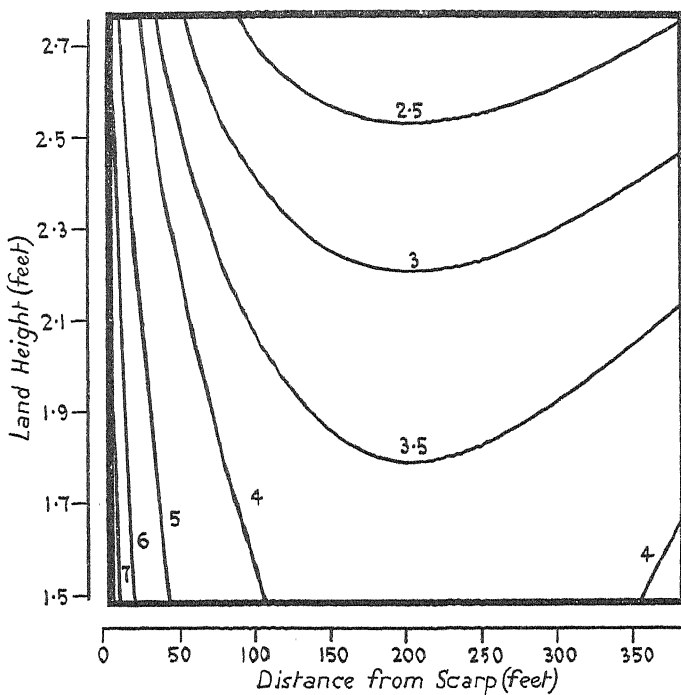


FIG. 2. Diagram showing contours of accretion surface, as related to Land Height and Scarp Distance over the observed ranges, from the *Armeria* of Line I. (Contour lines represent cm. accretion in 100 lunar months.)

difference that the contour lines would have been parallel and equally spaced though of the same general form as in the diagram presented.

The section parallel to the distance axis shows that accretion rate falls off very rapidly from the scarp inland, but that there is a minimum rate at approximately the middle of the marsh, after which accretion again increases slightly with further increase of distance. There can be no possible doubt of the reality of the rapid change in accretion rate near the scarp, but the positive sign of the correlation at the landward end of the line needs examination. For this purpose the partial correlation coefficient of accretion on distance, eliminating the effect of height, has been worked out for all points at a distance greater than 200 feet. The result shows that the correlation in this region is indeed positive, though it is not nearly close enough to reach the level of significance, e.g.  $r_{ad.h} = +0.250$  (5 per cent. = 0.514).

In the area of Line II, also at the Ynyslas end of the marsh, the partial

correlation coefficients are similar in sign and magnitude to those of Line I, though owing to the smaller number of points their significances are lower. The linear regressions in these two areas are also generally similar, but no marked improvement in the significance of the multiple correlation can be effected in the case of Line II by the use of a more complex regression function. The explanation of this is undoubtedly that whereas accretion rate increases with greater rapidity the nearer the scarp is approached, on this particular line no pure *Armerietum* points occur in the first 85 feet. As pointed out on p. 231, near the scarp, in transitional *Glycerietum* to *Armerietum*, rates of accretion twice as high as elsewhere on the line are again found. There is then no reason to suppose that the factors affecting accretion rate in the *Armerietum* are very different in these two areas.

In the case of Line III (Glandovey end) the partial correlations are again negative, though that with height is rather lower than in the preceding two lines and that with distance decidedly higher. The multiple correlation is highly significant, but again no marked improvement is obtained by introducing a curved regression on either variable. Fig. 3 shows the agreement between the calculated and observed values of accretion, together with the contour of the observed points; the fit is good except for certain points lying between 45 feet and 75 feet from the scarp, in which region the marsh is somewhat complex and local contours become relatively important. This irregular region is dealt with later (p. 242). Along Line III, then, the regression of accretion on distance would appear to be much more nearly rectilinear than is that in the region of Line I.

(2) *Glycerietum* and transitional *Glycerietum* to *Armerietum*.

Omitting the negligible negative correlation between accretion and height along Line IV (an area which has been shown to be highly irregular) and also the *Glycerietum* of Line I, there is in all other cases of vegetation below the *Armerietum* an *increase* of accretion rate with increasing height. Although this relation appears to be general it is unfortunately never sufficiently high to be significant, owing presumably to the small numbers of available points. In the early stages of salt marsh building the density of the vegetation appears to be an important factor affecting the rate of accumulation of silt. When *Glyceria* first colonizes the estuary a very loose open association is formed, which must be comparatively poor as a medium for the collection of silt. A certain amount is, however, accumulated, and the surface begins to rise slowly; in the meantime, the vegetation is rapidly becoming more and more dense, and although fewer tides now reach it, more material can be collected from those that do so. In this way the rate of accumulation increases for a time with the age and height of the marsh, but there comes a time when the rate of increase of vegetation density diminishes, and the effect of the ever decreasing number of tides



turns the scale in the opposite direction, so that thereafter the correlation between accretion rate and height becomes negative. The present results indicate that this reversal of the sign of the correlation coefficient occurs generally during the transition period between the *Glycerietum* and the *Armerietum*. In this connection the data for the transitional vegetation of Line II are interesting. Here the partial correlation has the comparatively low value of  $+0.399$ , and this is derived from points varying in height between 1.19 feet and 1.94 feet; but if all points at a greater height than 1.60 feet are omitted, the partial coefficient derived from the remainder is as high as  $+0.690$ , the 5 per cent. point being  $0.707$ . At these lower levels then, although there are only six degrees of freedom (9 observations), the positive correlation is to all intents and purposes 'significant'. The evidence points to the conclusion that in this area the reversal of the sign occurs approximately at the height of 1.6 feet above the level of the limit of vegetation.

The above remarks apply only to vegetation which has taken part in the primary building of the marsh, and not therefore to the *Glycerietum* of Line I, which is in a comparatively old area and in which all the primary colonizing *Glyceria* has begun to be invaded by *Armeria*. The eight pure *Glycerietum* observations were taken in growing-up pans or channels at varying levels, and there is here no connexion between land height and the density of the vegetation. The high and significant negative correlation is mainly due to one point; this is situated in a growing-up high level pan in the *Festucetum*. It is at a considerably greater height than any of the other observations, and has much the lowest rate of accretion, owing to the sparse vegetation together with the few tides reaching it. If this point be omitted, the correlation coefficient, as might be expected, becomes quite negligible, i.e.  $r_{ah.d} = -0.232$ .

The correlations between accretion rate and scarp distance require little comment; they are again negative, and that obtained from Line II is exceedingly high. As mentioned previously, here also are found greatly increased rates close to the scarp, as in the *Armerietum*. Presumably distance will have the same effect on accretion rate in all the vegetation zones.

### (3) *Festucetum* and transitional *Armerietum* to *Festucetum*.

Only on Lines II and III are there sufficient points higher than the *Armerietum* to render worth while the attempt to unravel the relations between the three variables studied, and it is certainly unfortunate that in both of these areas the same unusual combination of circumstances renders it impossible to determine the individual effects of height and distance on accretion rate. Along Line II, for example, the multiple coefficient reaches the high value of  $0.831$  (1 per cent. =  $0.732$ ), indicating that when height

and distance are taken into consideration the accretion rate may be very accurately predicted. The individual total correlations are of much the same magnitude (with height  $-0.8246$ , and with distance  $+0.8161$ ), showing that if either of these is considered alone the accretion rate is determined with an accuracy almost as great as when both are taken into account. This is brought about by the fact that height and distance are themselves extremely highly and negatively correlated ( $r_{dh} = -0.9494$ ), and therefore any other variable which is highly positively correlated with one, such as accretion rate in the present instance, must also be highly negatively correlated with the other. The partial correlations are quite insignificant in both cases ( $r_{ah.d} = -0.274$ , and  $r_{ad.h} = +0.187$ ). The conclusion to be drawn from the data, then, is that accretion rate is to all intents and purposes accounted for by land height and distance from the scarp, but owing to the very high correlation between these two last it is impossible to determine how the rate is related to them individually. On this line the transitional vegetation and the pure *Festucetum* have been treated together, since an examination of the data shows that there is no observable difference of behaviour between them.

By a coincidence, and for precisely the same reason, it is also impossible to analyse the only other *Festucetum* data, those of Line III. There would seem to be, however, no *a priori* reason for supposing that in the higher sward vegetation zones the rate of accretion varies with height and distance in a manner very different from that found in the *Armerietum*.

*Interpretation of 'Height' and 'Distance'.* From the above analyses it is seen that the more important factors determining the rate of vertical accretion at any point in a given area of the marsh are (1) density of vegetation, (2) height above mean sea level, and (3) distance from the river front of the marsh. The first two need little comment; factor (1) determines the efficiency of the point as a collector and retainer of the silt presented to it, while (2) determines the frequency and duration of the tides covering it. But the interpretation of factor (3) is less obvious; 'distance' is, indeed, probably a generalization which may suitably express the resultant effect of several subsidiary factors. In the most complete set of data dealing with scarp distance, that from the *Armerietum* of Line I, it is found that accretion rate is greatest near the scarp, and diminishes very rapidly inland, until at about the mid-marsh a minimum is reached; further inland again the rate increases slightly. In the absence of data to the contrary we may assume that with slight modifications some similar relation exists on the marsh generally. The very high accretion rate near the scarp would seem to be the result of two causes. Firstly, in the words of Yapp (7, p. 81), 'during high tides, as the channels become filled, the water tends to overflow their banks. When this happens, the coarser, heavier sediment is naturally deposited first, and so the banks of streams

and the larger channels often become slightly raised above the surrounding level. *This applies to the main river*, as well as to tributary streams and channels.' Thus as soon as it is flooded, the river edge will take first toll of the silt brought in by the tide. Such an effect must be general and act equally at both the eastward and westward ends of the marsh. But, secondly, there is at the Ynyslas (western) end a further cause of the high accretion rate along the scarp front; this is a supply of wind-borne sand from the dunes at the mouth of the estuary. Concerning this, Yapp says (p. 67) that near Ynyslas '*Festucetum* is developed mainly on a slightly raised strip of land along the northern or river edge of the marsh. Most of the blown sand is intercepted by this strip, hence the finer character of the soil of the *Armerietum*, which lies on its landward side.' An average mechanical analysis of the upper twelve inches of these two types of soil, as given by Yapp, is as follows:

	Coarse sand.	Fine sand.	Silt.	Fine silt.	Clay.
	%	%	%	%	%
Seaward raised strip (Lower <i>Festucetum</i> )	42·40	28·43	18·12	4·42	0·58
More landward soil ( <i>Armerietum</i> )	6·63	12·14	21·42	24·72	22·53

In the same place (7, Pl. XII, Photograph 1) appeared a photograph showing the raised seaward strip at the Glandovey end, standing exposed above the water while the marsh immediately behind it is submerged.

Another factor which is probably partially included in the term 'distance from the scarp' is the general slope of the land. Where the surface slopes comparatively steeply, so that during the ebb and heavy rain-storms water flows rapidly over it, much of the silt which was deposited at high tide must again be removed. This will affect open more than closed associations, and may well be one of the reasons why open *Glycerietum* does not accrete so rapidly as the later and denser vegetation, and the chief reason for the very slow accretion rates observed in bare pans, channels, &c. In the areas observed, land contours in one direction only are known, hence it is difficult to say how far this factor has been effective in the present instances. Along Line III, during the early stages of flooding, water gains access to the marsh, and later leaves it again, by way of a channel crossing the line at right angles, at approximately 160 feet from its river end. Owing to the general downward slope from the scarp to this channel, the amount of scour, which will depend very considerably on the amount of water flowing over the point, must be positively correlated with distance from the scarp over much the greater portion of the line; this may be the main reason for the relatively large effect of distance on

accretion rate in this area, leading in the linear regression equation to a high coefficient of Scarp Distance compared with that of Land Height. In the *Armerietum* of Line II also there is a high negative correlation

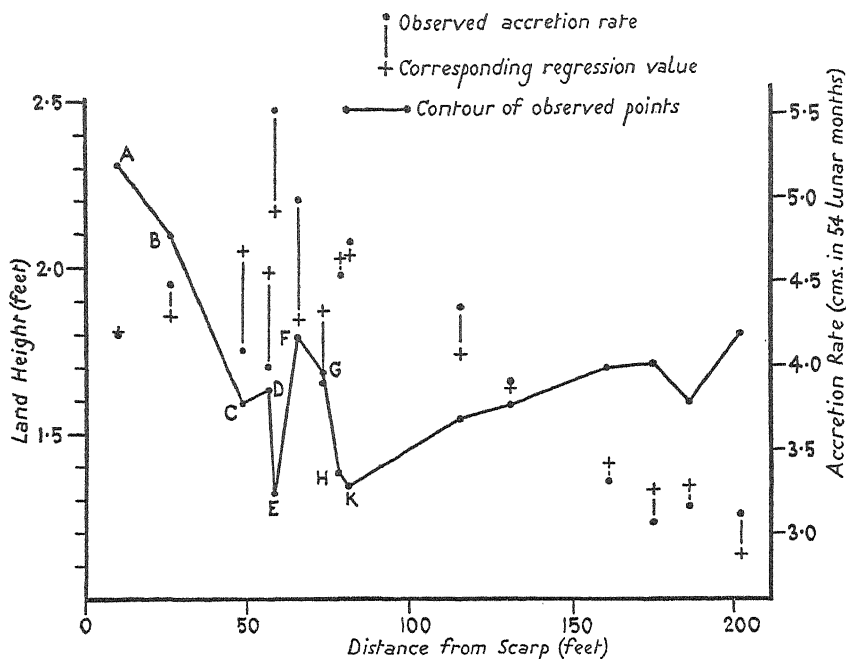


FIG. 3. Diagram showing, for the *Armerietum* of Line III, (1) contour of the observed points, (2) amounts of accretion observed at these points (cm. in 54 lunar months), and (3) accretion values calculated from the linear regression equation.

between height and distance, and again the relation between the coefficients of the regression equation is similar to that of Line III at the opposite end of the marsh, and not to that of Line I at the same end.

The effect of running water in the removal and subsequent re-deposition of sediment may be well illustrated in the irregular area of Line III, referred to above (p. 238). This area includes the points C-G in Fig. 3, where the fit of the regression equation is poor. On the marsh, between C and D, there is a *Festucetum* hummock rising to a height actually greater than that of B; C and D lie on the comparatively steep slopes of this hummock. On its river side the land falls slightly beyond C to a hollow containing *Glycerietum*, and on the landward side to a channel a few inches beyond the point E. It appears probable that summit points will have little sediment removed from them, since the amount of water running over the surface will be minimal. On a uniform slope, the lower the point the greater will be the amount of silt removed; where the slope is not uniform the rate of flow and of surface erosion will vary with the angle of

slope; while in the hollows themselves the material carried down may once more be trapped and accumulate. On these assumptions C and D, lying low down on steep slopes, may be expected to have abnormally low rates of accretion, and it is actually found that the observed rates are much below the expected values when these factors are not considered. On the contrary, the observed rate at E, lying in a deep depression, is much higher than the regression value. Similarly, it may be mentioned that the *Glycerietum* in the hollow on the river side of C has an abnormally high rate: 7.25 cm. per 50 months, compared with 4.1 cm. in the *Armerietum* at C, from which it differs but slightly in Scarp Distance and Land Height.

Again, F is a summit point, and may be expected to have a rather higher value than normal;<sup>1</sup> G lies on a moderate slope, and has a low value: while H lies at the foot of the slope (a channel running immediately on its landward side), apparently near the point where removal and re-deposition compensate for each other.

Exactly how far the curved regression of accretion rate on distance, obtained from Line I, is applicable to the marsh as a whole is questionable, but it is highly suggestive that a cross-section of the marsh usually reveals a relationship between present height and distance very similar to this regression.

This is clearly shown in Fig. 4, where the observed contours of the four lines are presented to different horizontal scales. The general similarity between the sections in the areas of Lines II, III, and IV is obvious, as is their common resemblance to the three accretion regression curves presented on the same diagram. The broken regression line is the one derived from the *Armerietum* data of Line I when the first power and the logarithm of Scarp Distance are considered, i.e. a cross-section of Fig. 2. A regression of this form has the disadvantage that it must of necessity give a value of + or - infinity at the scarp itself; hence the regression equation involving Land Height and the first and second powers of Scarp Distance has been calculated:

$$\text{Accretion} = -0.97792 (\text{Land Height}) - 0.028459 (\text{Distance}) \\ + 0.000061949 (\text{Distance})^2 + 7.9818.$$

The fit of this equation is not so exact as that given by the one involving logarithms, but it is very good, as is evidenced by the high multiple correlation coefficient, i.e. 0.8865. The extra goodness of fit of this equation over that of the rectilinear regression is also very highly significant:

$$s = 1.461 \text{ (1 per cent.} = 1.022).$$

<sup>1</sup> This would not seem to account entirely for the high rate observed here; possibly a more important factor in this particular case may be that the species characteristic of Lower *Festucetum* are becoming numerous; the vegetation is in a very late *Armerietum* stage and unusually dense (cf. 7, p. 72).

This particular regression on Scarp Distance is plotted as the curved dotted line in the figure.

The third regression shown there (continuous curve) is the one obtained

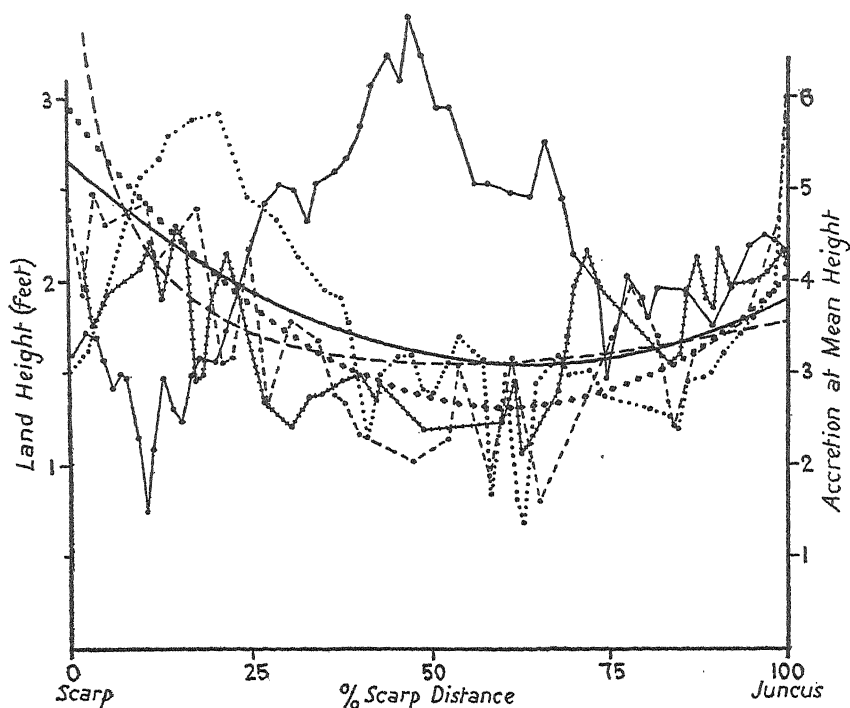


FIG. 4. Diagram showing (1) observed contours of Lines I-IV, to different horizontal scales, and (2) regression of accretion on Scarp Distance derived from data of Line I. For explanation see text.

Contours: Line I, continuous; Line II, dotted; Line III, broken; Line IV, hatched.

Regressions: (1) From *Armerietum*, first power and logarithm, broken; (2) from *Armerietum*, first and second powers, dotted; (3) from all points, first and second powers, continuous.

by introducing the first and second powers of Scarp Distance into the equation representing not merely the thirty *Armerietum* points of this line, but the total 53 points, all zones being considered together. By this inclusion no marked change in the shape of the regression is indicated, but it should be remarked that when the line is considered as a whole the goodness of fit of the curved regression is not significantly better than that of the rectilinear.

The main difference between the general land contour of Lines II, III, and IV on the one hand and on the other the general shape of the regression curve for accretion rate, derived from the best data, those of Line I, is that the scarp end of the section is relatively slightly low, while the landward end is relatively rather high. If, as is probable, colonization of the

marsh began normally at the landward end, and gradually encroached further into the estuary (cf. 7, p. 81), this factor in conjunction with the regression of accretion on distance will account almost entirely for the present general surface configuration. At the extreme scarp end the effect of the periodic waves of erosion and re-building can also be seen in the lowering of the section. Only in the case of Line I—the line from which the regression is obtained—is there any marked departure from this form. This is probably the result of an unusual initial colonization in the area, age of the marsh not necessarily increasing with increasing distance from the river.

(e) *Rate of Change of Vegetation Zones.*

From the regression equations the time taken by a given point to accrete a definite amount may be calculated. This has been done for the *Armerietum* of Lines I and III. Along Line III the approximate height limits of the association are 1.3 and 2.3 feet, and the following periods are obtained, from the linear regression equation, for the points under consideration to rise this particular foot, i.e. to traverse the complete association :

Point.	Time.
10 feet from the scarp	26 years.
Mid-marsh (100 feet)	33 years.

Values for Line I, also calculated from the linear equation, are given below ; it should be noted that here the vertical range of the *Armerietum* is slightly greater than in Line III (1.5-2.75 feet).

Point.	Time.
10 feet from the scarp	72 years.
Mid-marsh (200 feet)	85 years.

For a direct comparison with the area of Line III the corresponding times taken to rise the first foot in this zone may be used : 53 and 61 years respectively. It appears that the general rate of rise is just about double at the Glandovey end of the marsh (head of the estuary) what it is at the Ynyslas end, but owing to the greater vertical range of the association in the latter region the time taken for any point to pass through the zone is more than double that taken near the former. The other vegetative zones show similar differences at the two ends of the marsh.<sup>1</sup>

<sup>1</sup> There is no doubt as to the reality of the difference in the observed accretion rate at the two ends of the marsh. If the observed increases in height in the *Armerietum* of Lines I and III—reduced to an equal time interval—are compared by means of 'Student's' test, a value for 't' is obtained of 9.83, 'n' being 43 (i.e. 1 per cent. = 2.7). This very high value would be higher yet were it not that the variance within the two groups is much increased by the variations in land height and scarp distance ; but this consideration is offset by the fact that the difference between the means should be slightly lower than the value used, owing to the *Armerietum* being on the average rather more advanced on Line I than on Line III, i.e. on Line I the points are relatively higher and therefore tend to accrete less rapidly than on Line III.

A better estimate of the exact conditions prevailing in the region of Line I may be obtained from the equation involving the logarithm of distance. The times taken to traverse the *Armerietum* are as follows:

Point.	Time.
10 feet from scarp	46 years.
40 " " "	67 "
Mid-marsh (200 feet)	98 "
Landward limit (380 feet)	87 "

From the regression equations for the *Glycerietum* and *Festucetum* could also be calculated corresponding time periods for those associations, so that a figure could be given representing the time which has elapsed since any given point was first colonized by vegetation; but since figures based on insignificant regression equations might be grossly misleading, no attempt has been made to determine these times. Suffice it to say that each subsequent association must take a longer period to complete its history than does the *Armerietum*, in which accretion rate attains its maximum rapidity.

(f) *Morphological Changes Induced by Rapid Accretion.*

As has been stated, a fifth line was laid down on the very sandy area near the river end, and on the east side of the great breakwater in the Glandovey area of the marsh. In this specialized region almost all the observed points are in the *Glycerietum*, but, as indeed is not surprising, no significant correlation coefficients can be obtained, though there is again strong evidence that higher parts accrete more slowly than lower unless the latter are very open and therefore lacking in binding power; and also that accretion increases as the river or a large channel is approached.

The area is remarkable for the magnitude of its general rate of vertical growth, rates up to, and even slightly exceeding, 8 cm. in 16 months having been observed, i.e. nearly 0.5 cm. per tidal cycle. Such rates naturally have a profound influence on the morphology of the plants growing there, which must keep pace with the vertical growth; and it appears that most of the energy of the *Glyceria* is used up in this, very little tillering being found (see Fig. 5, A). This is in strong contrast to the conditions obtaining some 50 feet inland, and at approximately the same height, where the rate of accretion is only 2.5 cm. per 16 months, though this rate is high compared with those in the areas previously discussed. Here the plants manage to keep their aerial portions above ground with moderate ease and tillering is abundant (Fig. 5, B). Where accretion rate is excessive the main stolon may be traced to a considerable depth underground, with nodes at intervals of approximately 1 cm.; in the case of the plant drawn, the stolon extended more than 12 cm. below the soil. The one tiller present was completely buried and dead.

The close tufted habit of growth of *Glyceria*, seen in B, is characteristic



of the species when growing under drier conditions, as on a sandy or well drained soil, and normally towards the upper limits of the association. In wetter places it is much less tufted, forming long creeping shoots. The

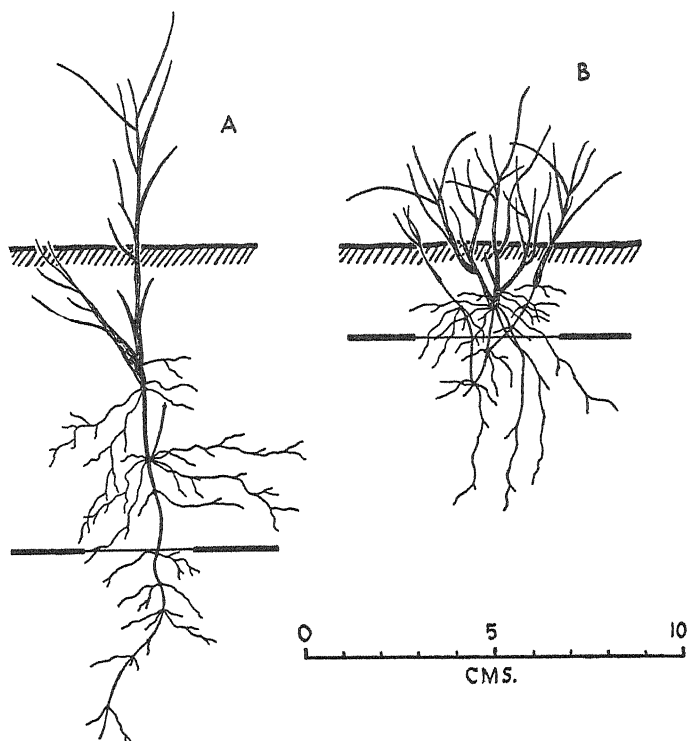


FIG. 5. Typical *Glyceria* plants from Line V (after Yapp). A, plant from region of very high accretion rate; B, plant from region of more moderate accretion rate. Present surface level and surface level 16 months previously are indicated.

tufted form must be more efficient as a collector of silt than the creeping, and hence the general positive correlation between accretion rate and land height in associations below the *Armerietum* will normally be accentuated by this change in the habit of growth. This will account for the prominence of early *Armerietum* hummocks on young marsh generally, since these must grow vertically much more rapidly than the open *Glyceria* surrounding them. The young *Armerietum* in the neighbourhood of Line V, though raised, is not so abruptly hummocky as is that in the more or less waterlogged regions of Lines III and IV; and the *Glyceria* for the most part is less open. In all probability the better drainage of Line V allows *Glyceria* more rapidly to assume the tufted form, and therefore to accrete more rapidly. Under these conditions the early *Armerietum* will not rise at a much more rapid rate than the *Glycerietum*, and the marsh will therefore be but slightly hummocky.

In general it may probably be said that where drainage is deficient an association may persist at higher levels than normal ; in other words, poor drainage retards while good drainage accelerates the normal succession on a salt marsh.

(g) *Factors Affecting the Rate of Accretion.*

The main factors determining the rate of vertical accretion at any point which have been discussed in the preceding sections may be conveniently summarized as follows :

A. *Supply of material.*

1. General position in the estuary. The rate is much higher near the head of the estuary than the mouth, due to differences in the silt burden of the water, flocculation of fine particles in suspension in fresh water on meeting salt water, &c.
2. Distance from river front, or a large channel, as affecting (a) the supply of material in aqueous suspension, and (b) the supply of wind-borne sand, particularly near the mouth of the estuary.
3. Height above mean sea level as determining the frequency and duration of submersion.
4. Presence of obstacles (as a breakwater) which affect tidal currents locally, and in the lee of which water- and air-borne particles may accumulate.

B. *Collecting and retaining efficiency.*

5. Density and character of vegetation. This affects the collecting efficiency by determining the resistance to flow (cf. 4), and the collecting area ; while the power of retention must be closely related to the binding power of the vegetation.

C. *Subsequent changes of deposits.*

6. General slope and topography of the marsh at the point, and
7. Density and character of vegetation, as determining the amount of silt removed or re-deposited by the ebb-tide currents, heavy rains, &c.

3. RATE OF FORMATION AND EARLY CHANGES OF THE MARSH.

Other types of change on the Dovey salt marshes will be dealt with only briefly, as they have not been observed sufficiently widely or over a long enough period of time to enable them to be expressed in exact quantitative terms.

The rate of horizontal spread of vegetation over the bare silt of the

estuary has been studied by observing for a period of several years a small isolated area of young marsh at the Ynyslas end. The area is one situated at the limit of vegetation towards the river, and has been surveyed on three occasions: (1) July 1922, (2) June 1925, and (3) April 1930 (see Figs. 6–8). In 1922 it consisted of a main 'island' of comparatively old marsh chiefly in the *Armerietum* stage, together with, on its landward side, some half dozen smaller islands sufficiently old to be partly colonized by *Armeria*, and also a large number of small islets of *Glyceria*; very many of these last consisted of single plants. The main island formed a comparatively simple hummock, the higher portion of which had been completely colonized by the *Armerietum* flora, leaving a narrow peripheral band of *Glyceria* which was still spreading laterally.

The river side was sufficiently advanced to have suffered considerable erosion, resulting in an escarpment some 12 inches high, but there was no secondary marsh forming below this. Crossing the main mass were two sheep-tracks in which *Armeria* had been unable to obtain a footing, or alternatively was insufficiently resistant to the continual wear.

In the 1925 survey many interesting changes are recorded. Since 1922 considerably more erosion had taken place at the river side, removing all traces of the peripheral *Glycerietum* in this region, but secondary sward had not yet begun to develop at the silt level. The most marked change was at the landward side, where the small *Glyceria* islets had spread laterally over the silt to such an extent that the majority had coalesced with similar islets or with the main mass. Perhaps the most striking feature brought out by the two surveys is the persistence of even minute *Glyceria* plants once they have rooted on the bare silt; in almost every case the individuals mapped in 1922 had survived until 1925. In addition, the main island had extended laterally (except at the river side as mentioned before) owing to the colonizing power of the *Glyceria*. The *Armerietum* had not extended in a manner at all comparable to that of the *Glycerietum*; in fact very little extension of the masses present in 1922 had occurred, and but four new areas appeared.

The 1930 survey shows a continuation of these changes. Erosion at the river end had advanced considerably, and much of the fallen vegetation had succeeded in re-establishing itself at the lower level, so that secondary sward formation had begun (cf. 7, p. 82). Almost all the *Glyceria* islets of 1922 had extended to such a degree that they formed a continuous sward with the original main mass, though much of the vegetation in this area was yet very open. New islets were still appearing on the silt at the side away from the river, where presumably broken bits of *Glyceria* washed by the tides were left to establish themselves as new growth centres. The shelter afforded by the main mass would seem to be an important factor in this process, and the surveys show a marked difference between the rate of

spread away from the river behind the older marsh, and that in any other direction. This difference appears to be due entirely to the continual formation of new colonizing centres in this direction and in no other; extension in a direction parallel to the river is dependent almost entirely on the gradual growth of the existent periphery. In considering the spread of a plant community by vegetative growth over a horizontal area under uniform conditions it seems clear that the rate of colonization of the two dimensional surface must be a function of the length of the growing edge, i.e. a one dimensional measure. Hence enormously increased rates may be expected where the length of the growing edge is much increased by the formation of fresh centres of colonization. In the present instance, owing to there being but three surveys of the area over a period of rapid growth and coalescence, it is of little use to attempt the laborious determination of the extent to which the difference between the rate of spread away from the river, and that in the direction parallel to it, may be ascribed to this cause and how far to others—such as the protection afforded by the main mass, tidal currents, &c.

The 1930 survey shows again very slight increase in the total area occupied by *Armeria*; indeed, some of the areas appear definitely to have shrunk slightly, while three of the four new centres noted in 1925 had disappeared entirely, possibly because of the very low level at which colonization was attempted.

It is evident therefore that lateral extension of *Armeria* is an exceedingly slow process when compared with that of *Glyceria*. On the other hand, all the older areas of *Armerietum* marked on the surveys stand out clearly in the field, not only because of the different appearance of the vegetation, but because they form pronounced hummocks on the lower *Glycerietum*. Each hummock presents a weak surface to the tides, and may even suffer erosion with the formation of a miniature scarp. This is shown well on the surveys in the *Armerietum* mass situated 32 feet from the southern boundary of the survey, and 17 feet from the western; in 1922 it was an ordinary hummock, sloping off gradually on all sides, but by 1925 the river side had yielded to wave action, forming an escarpment as much as 5 inches high. These results then accentuate the difference in the parts played by *Glyceria* and *Armeria* as marsh builders, if further stress were needed on this point; *Glyceria* is the primary colonizer and is entirely responsible for the lateral spread of the marsh; this is accomplished rapidly because of the stoloniferous habit of the plant, and may be greatly accelerated by the ease with which new centres of colonization are formed. *Armeria*, on the other hand, is primarily concerned with the vertical raising of the young marsh, its habit being well adapted to change in this direction.

Though the area occupied by *Armeria* changed very little between 1922 and 1930, the survey of the latter year shows that a large number of

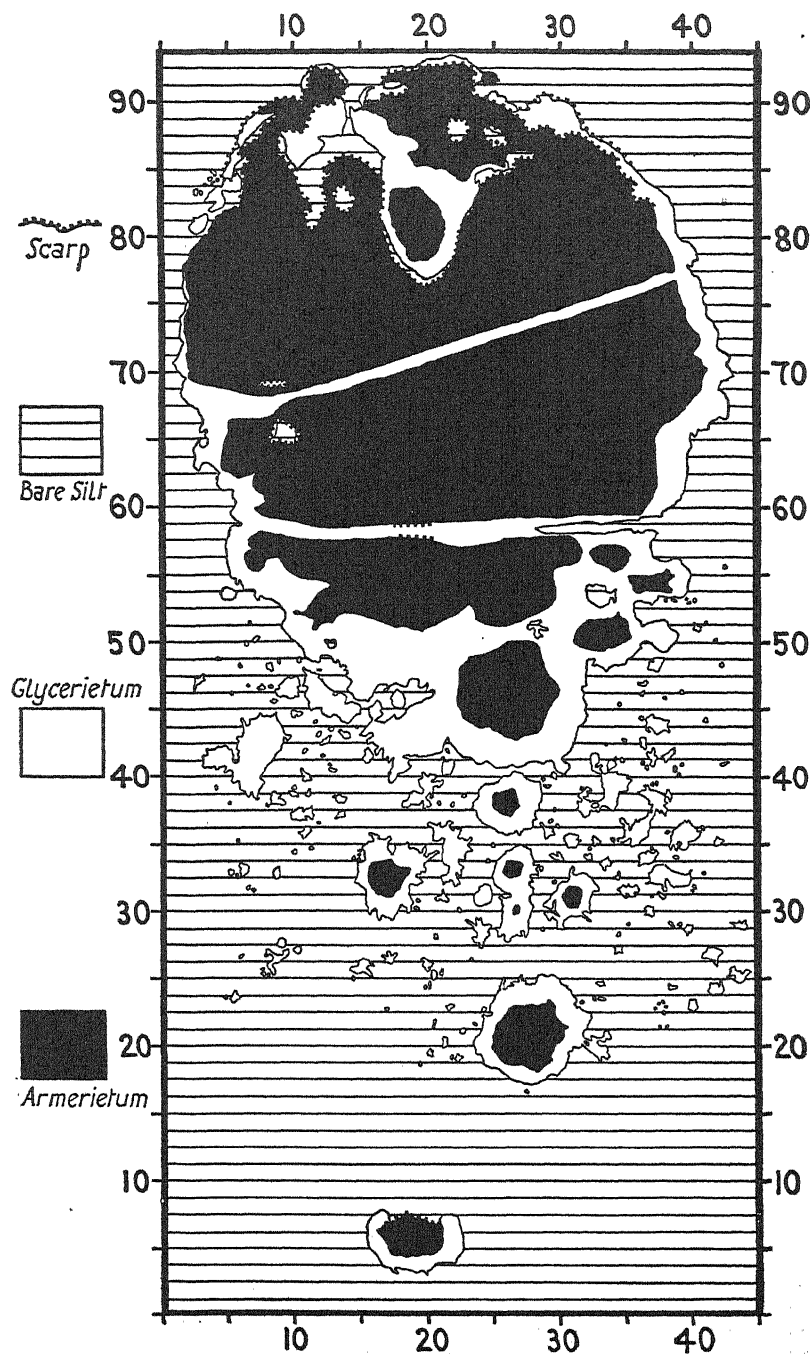


FIG. 6. Survey of area of young marsh in July 1922 (surveyed by U. C. Slane and F. J. Richards), for comparison with Figs. 7 and 8. Distances in feet.

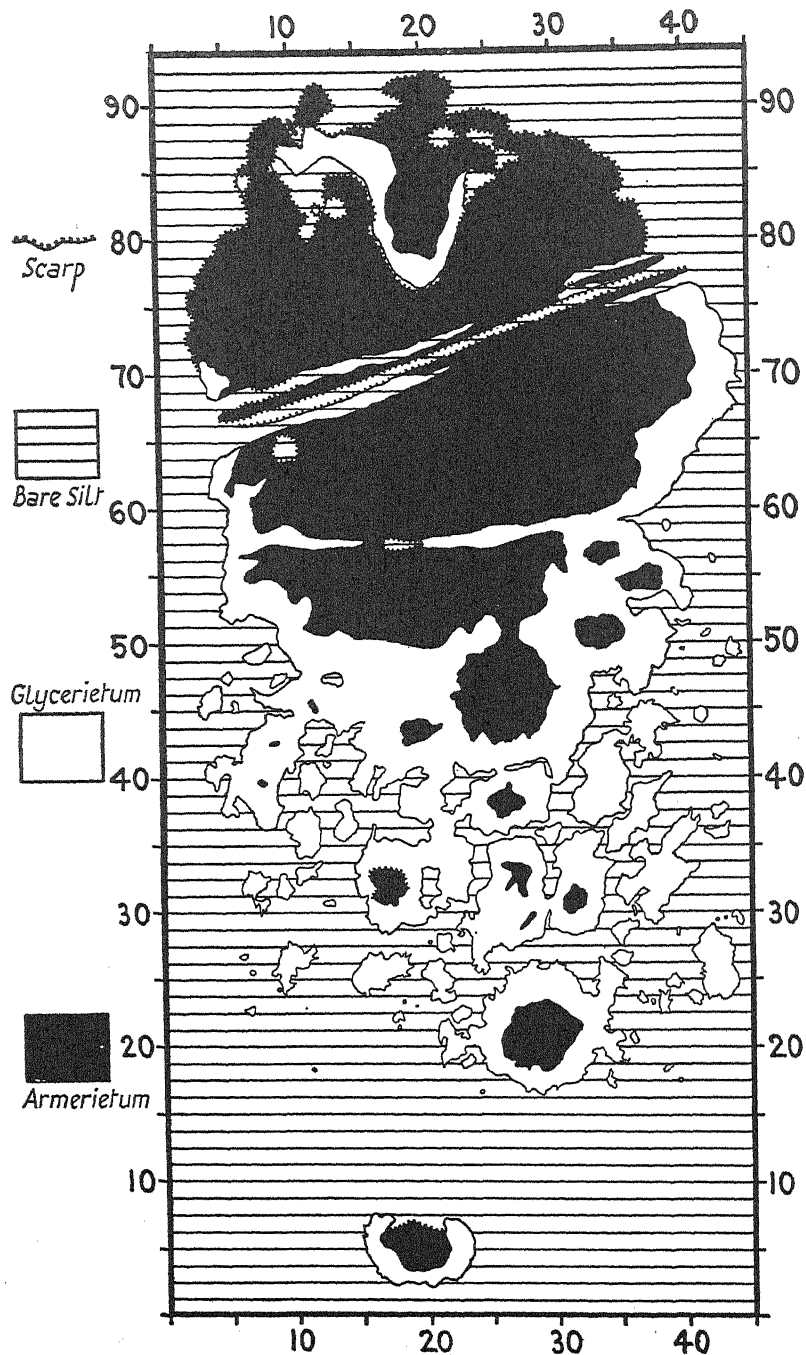


FIG. 7. Survey of area of young marsh in June 1925 (surveyed by E. W. Rogers and F. J. Richards), for comparison with Figs. 6 and 8. Distances in feet.

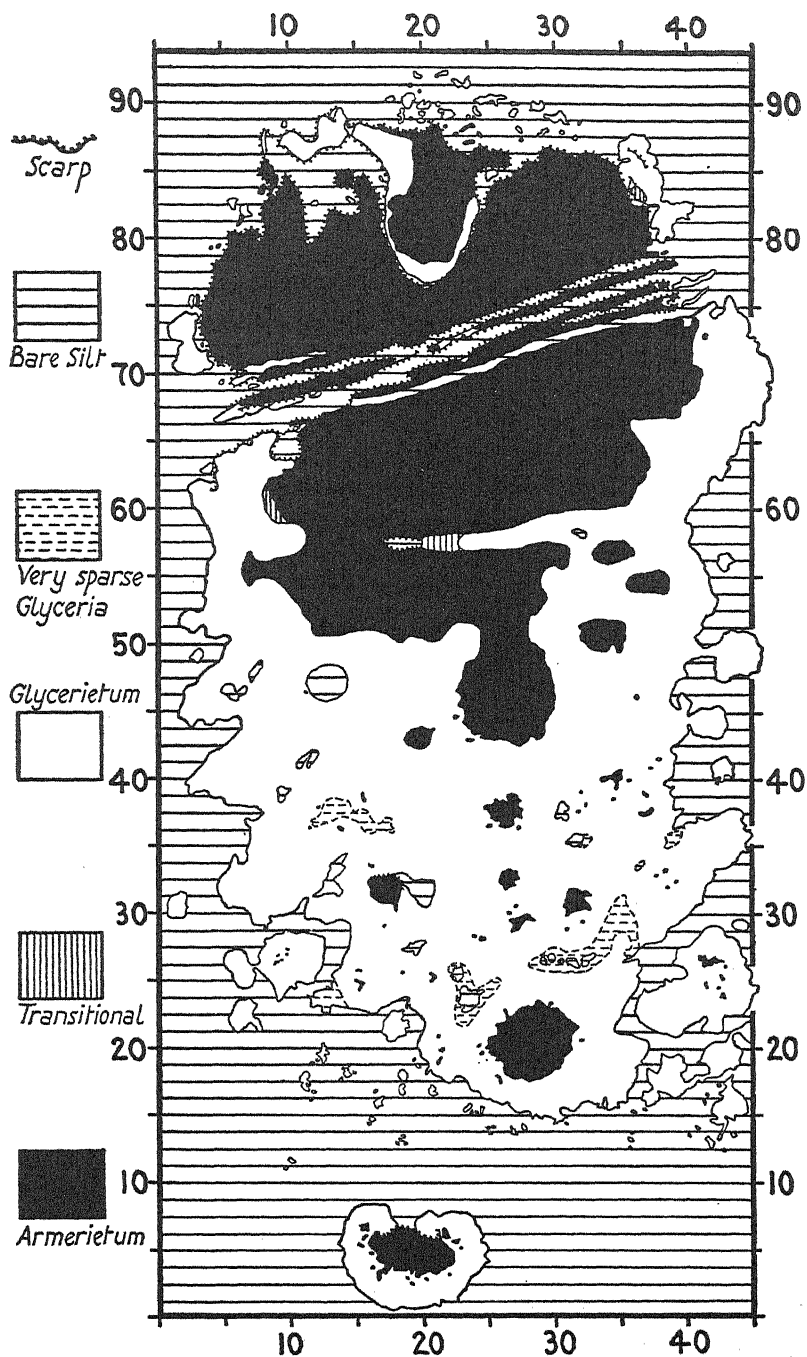


FIG. 8. Survey of area of young marsh in April 1930 (surveyed by L. K. and F. J. Richards), for comparison with Figs. 6 and 7. Distances in feet.

seedlings had obtained a footing on the more raised portions of the new marsh, and these appeared to be doing well. Of course it is probable that many of these will die out, as did the three new colonizations of 1925 noted above; but it is interesting to note that in several cases *Armeria* had been able to obtain a footing, by the summer of 1929, on areas which must have been unoccupied by vegetation in 1921.

Finally, it may be pointed out that a new method of pan formation, so far as the Dovey Estuary is concerned, is revealed in these surveys (cf. 5 and 7). In 1922 two sheep tracks crossed the area. By 1925 one had been deserted while the other, by frequent use, had grown more complex. The deserted one proceeded to grow up and become indistinguishable from the surrounding sward, with the exception of a small hollow in which water accumulated. The *Glyceria* in this hollow had disappeared by 1925, and by 1930 a small pan, 3 feet by 1 foot, had developed; it will doubtless remain a permanent feature of the marsh. This is the first and only case observed by Yapp of a pan originating directly on the sward instead of on bare silt. Warming (4) had stated that such methods of pan formation may occur, as, for instance, where the turf has been weakened or destroyed by putrefying masses of algae or *Zostera*, or by the treading of cattle; but these were not previously accepted by Yapp (7, p. 87, and 5, p. 19) at least in so far as the Dovey marshes were concerned. It is clear that in this estuary such methods are not usual and are quite unimportant in the history of the marsh.

#### 4. SCARP EROSION AND FORMATION OF SECONDARY MARSH.

These phenomena have been observed by surveying selected areas. Two areas at the Glandovey end of the marshes were chosen in July 1922, one in which secondary marsh formation was in its first stages and one in which it was already well established. These areas were re-surveyed in June 1925, and again in April 1930; the surveys of 1922 and 1930 are presented in Figs. 9 and 10. As again quantitative figures for rates of change cannot be given with any precision, only the more salient facts brought out by these surveys will be indicated. In the case of the area in which secondary marsh formation is just beginning, and scarp erosion is presumably at its most active stage (Fig. 9), it is readily seen that this latter is a very slow process. The primary marsh, which forms an escarpment roughly 20 inches in height, has been cut back approximately 11 inches during the period between the surveys, i.e. rather less than 1.5 inches per annum. In the case of scarps which are partially protected by a well-established secondary marsh (Fig. 10) the rate of erosion is apparently considerably lower yet. The chief difficulty in obtaining such rates in a comparatively short time lies in their discontinuity. The great binding



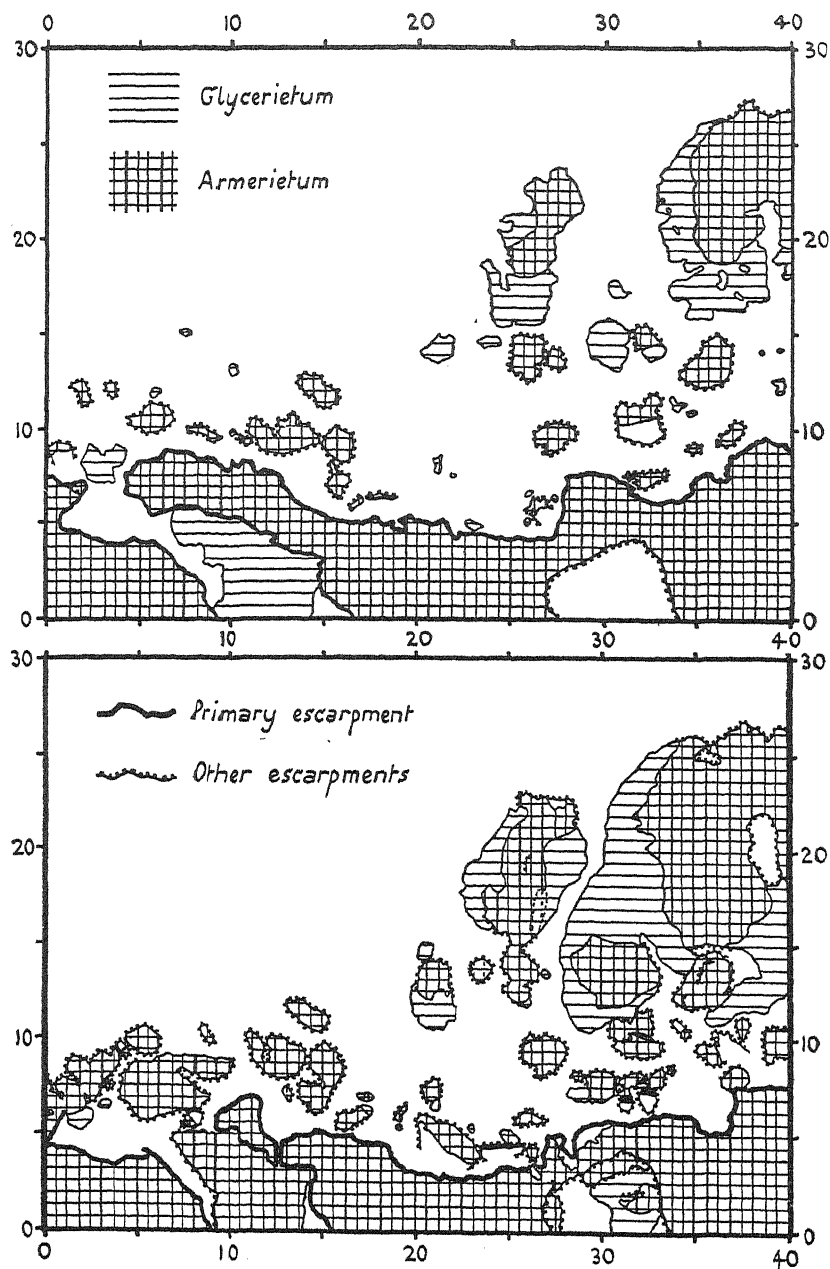


FIG. 9. Successive surveys of area of young secondary marsh with primary escarpment. (1) July 1922 (surveyed by U. C. Slane and F. J. Richards). (2) April 1930 (L. K. and F. J. Richards). Distances in feet.

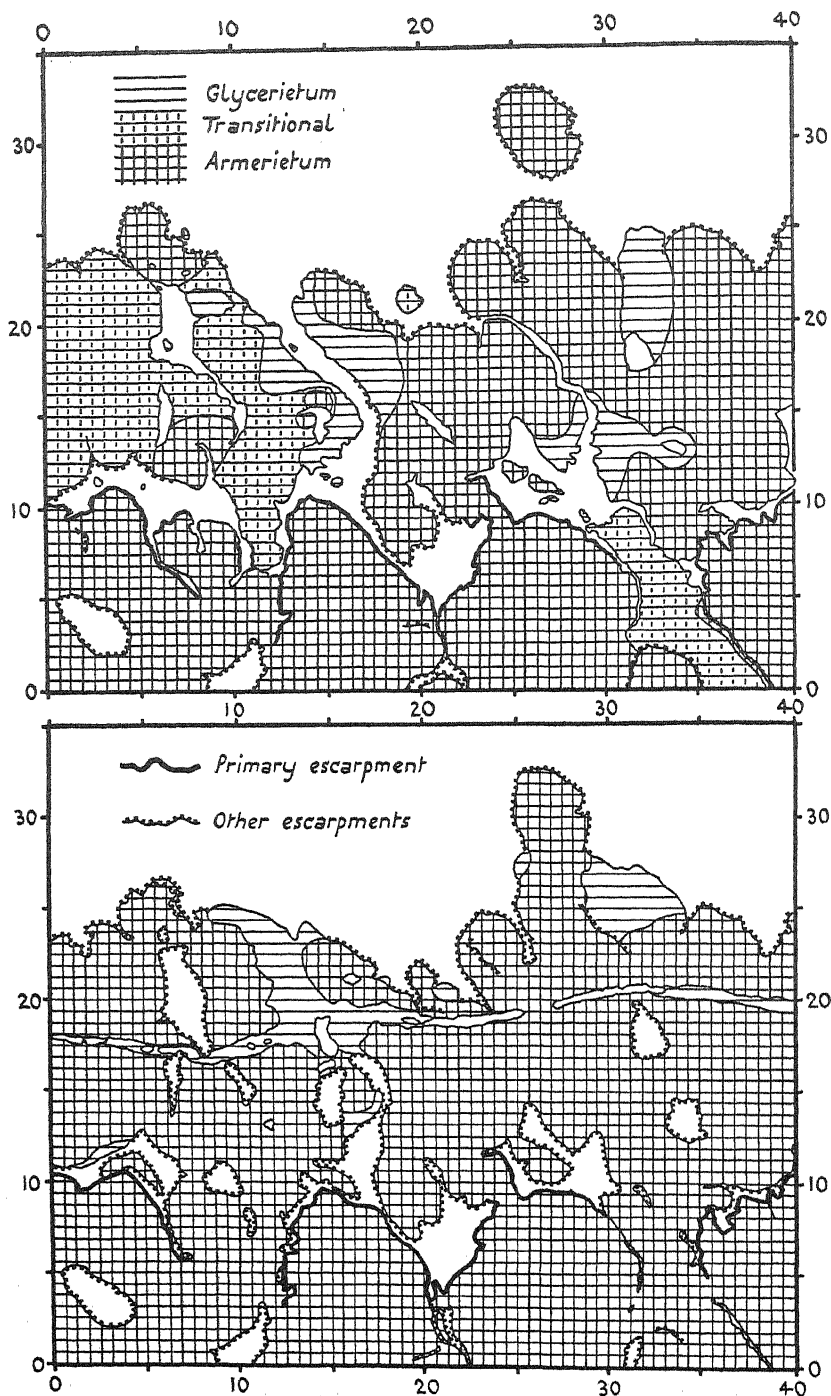


FIG. 10 Successive surveys of area of well-established secondary marsh, with primary escarpment. (1) July 1922 (surveyed by U. C. Slane and F. J. Richards). (2) April 1930 (L. K. and F. J. Richards). Distances in feet.

power of the roots of the sward plants results in the looser silt of the lower part of the scarp being eroded more rapidly than that of the upper layers, and this condition is accentuated by the fact that the foot of the scarp is exposed to tidal scour more frequently and for longer periods than the upper portion. Undermining is therefore the general rule, with the consequence that the primary sward itself remains almost unaffected by eroding influences for long periods of time, until a comparatively large mass breaks away and falls to the bare silt below. Sward erosion then, which is indicated on the surveys, is a discontinuous process, though erosion at the foot of the scarp must be more nearly continuous. As was pointed out by Carter (1), in this estuary ordinary tides do not play much part in scarp erosion; it is chiefly in times of storm or very high tides that the escarpments suffer most denudation. According to her observations, marginal erosion is most rapid in mid-winter and mid-summer (January and July).

The above data were obtained from the Glandyfi end of the marshes; the only data concerning the Ynyslas end are contained in the three surveys previously considered (Figs. 6-8). Owing to the complicated structure of the scarp in this area, a representative figure for the rate of erosion is even more difficult, but the process would appear to be between two and three times as rapid as in a similar area near the head of the estuary. This is due not only to the nearness of the open sea, but probably also, as pointed out by Yapp (7, p. 88) in connexion with pan erosion, to the more sandy nature of the marsh in this region. Even here the rate of erosion is very slow and somewhat reminiscent of the rate of pan enlargement (5).

Much of the fallen turf is gradually disintegrated, but some succeeds in re-establishing itself at the silt level and by subsequent lateral growth in re-forming the marsh (secondary marsh). The initial establishment appears to take considerable time, as may be seen by a comparison of the two surveys in Fig. 9. Some of the fallen vegetation blocks of 1922 had entirely disappeared by 1930; some had managed to exist without showing marked increase in size; while others had begun to spread laterally on the silt. The most important factor in determining whether re-colonization from fallen turf shall immediately proceed or not is the presence or absence of *Glyceria*. If the latter is abundant horizontal growth begins and a growing edge is soon formed. When this is well established the formation of a more or less continuous sward is quite rapid, as may be seen in the area at the right of Fig. 9; in this area too, the beginning of a second erosion scarp may be seen some 20 feet nearer the river than the primary scarp. Should *Glyceria* be absent from the fallen block the *Armeria*, *Festuca*, &c., may or may not persist, but in no case will appreciable lateral growth be made. The block may retain its individuality for many years without any marked change in size and shape, until finally it becomes

engulfed by *Glyceria* spreading from other areas; still later, when the general level of the secondary marsh has been raised and conditions suitable for the vigorous growth of species characteristic of the higher associations have again been realized, these clumps may become important as centres of colonization for those species. An example of such a block being surrounded by *Glyceria* from outside sources may be seen in Fig. 9, some 13 feet from the bottom and 4 feet from the right edge.<sup>1</sup>

Later stages in secondary marsh formation are very similar to those of primary formation and are indicated in Fig. 10. Lateral extension is almost complete and many pans have been formed, mainly at the foot of the primary scarp. The secondary marsh has a total width of 15–20 feet, and the formation of a secondary scarp is almost complete.<sup>2</sup>

#### SUMMARY.

1. The rate of vertical accretion in five selected areas on the Dovey salt marshes has been investigated over a period of eight years. In the examination of the data the statistical methods of correlation and regression have been used, since they present the most reliable and efficient means of extracting the relevant information.

2. The rate of accretion is found to differ in the different sward associations. In general it is greatest in the transitional vegetation between the *Glycerietum* and the *Armerietum*, being lower in the pure *Glycerietum*; it also decreases gradually throughout the later history of the marsh.

3. The rate at any point has been correlated with the height of the point above sea level. In the *Armerietum* and later associations the correlation with height is negative—accounting largely for the decreasing accretion rate through the successive vegetation zone—but in the stages prior to the *Armerietum* it is positive. In this case the most potent factor is not the height of the point, but the density of the vegetation. This increases with age and therefore with height; consequently the silt collecting power of the point increases with height.

4. Accretion rate has been related also to distance from the main river front. In general a high and negative correlation coefficient is obtained. From the best data concerning the relationship it appears that near the limit of the marsh towards the river accretion rate decreases very rapidly with increasing distance, but further inland changes more gradually, and may even increase slightly with distance towards the landward side. Such a relationship agrees well with the most usual form of the cross-section of the marsh and certainly accounts largely for this.

<sup>1</sup> In Fig. 9 the large pan at the bottom edge is growing up owing to drainage; an outlet has been cut in the landward side of the pan, i.e. not in the surveyed area.

<sup>2</sup> A sheep track was made across the secondary sward between 1925 and 1930.

5. The probable significance in these correlations of the variables 'Height' and 'Distance from the river front' is discussed.

6. From the regression equations the time taken for the surface of the marsh to rise a given amount, or to pass completely between the limits of any vegetation association, may be calculated; this has been done for the most significant data.

7. Where the rate of vertical accretion is very high it exerts a profound influence on the morphology of the plants concerned.

8. The main factors recognized as affecting the rate of accretion on the Dovey marshes are summarized and tabulated on p. 248.

9. The rate of spread of *Glyceria* over the estuary silt, and its subsequent invasion by *Armeria*, have been studied by means of surveys. *Armeria* has obtained a footing in marsh which cannot have been in existence for more than 8 years.

10. Surveys illustrating the rate of scarp erosion and of secondary marsh formation are presented. Scarp erosion is a slow process in the Dovey estuary, 1.5–3 inches per annum being usual; the rate depends apparently on position in the estuary.

In conclusion I desire to record my gratitude to the late Professor Yapp for his earlier guidance and stimulus; to Professor V. H. Blackman and Dr. F. G. Gregory for much valuable criticism; to all those who have aided in the amassing of data, and particularly to my wife without whose assistance the work of 1930 would have been impossible; to Mr. W. H. Mappin, of Ynyshir Hall, the owner of the marshes; and to the Civil Engineering Department of the University of Birmingham for the loan of surveying instruments.

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# The Cytology and Development of *Ascophanus Aurora*.

BY

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AND

H. S. WILLIAMSON.

With Plates VI and VII and three Figures in the Text.

*ASCOPHANUS AURORA* (Crouan) Boud. is a small, discomycetous fungus occurring not uncommonly on sheep pellets and giving rise to orange-brown apothecia with protruding asci and hyaline spores. It grows readily in culture and fruits well, especially on agar made up with a decoction of sheep dung to which chopped grass has been added. The fungus is homothallic and monoecious, ascocarps appearing in single spore culture. The type of growth is variable, sometimes the mycelium is sparse, extending quickly to the edge of the dish and giving scanty fruits, while in other cultures from the same source and on identical media large numbers of ascocarps appear in successive zones before the agar is half covered. It was mainly from rich growth of the latter type that material for examination was obtained. On it sexual branches appear six or seven days after the germination of the spores and mature fruits in fourteen days. Illumination is not essential to the development of the fungus which grows and fruits as readily in darkness as in light.

The spores germinate without a period of rest, the best results being obtained when they are placed for three hours in an incubator at 48° C. and transferred to an incubator at 25° for the remainder of their development. Material was fixed while still in the incubator, care being taken not to disturb it or subject it to changes of temperature before killing. Series of fixations every hour from noon to 10 p.m. gave abundance of critical stages. Flemming's strong solution diluted with an equal quantity of water was used as a fixative throughout the investigation.

For morphological examination thin slices of agar bearing the fungus

were stained with erythrosin (10) and mounted in glycerine jelly. Owing to the small size of the fungus a one-twelfth objective had to be employed, it was therefore necessary to ensure that the film of jelly above the specimen was very thin. Sections for cytological work were cut 7 to 10  $\mu$  thick and were stained for the most part in Heidenhain's haematoxylin and erythrosin in clove oil; they were mounted in Sira medium.

Germination was studied in microtome sections, of ascocarps with ripe spores which had been placed in drops of Barnes' medium (10) on sheep-dung agar, incubated at 48° C. from 5 to 8 p.m. and left at 25° C. till the following morning when they were fixed without removal from the incubator. By that time many of the spores had become multinucleate and the most advanced showed narrow germ tubes. As in *Pyronema confluens* (11), the nuclei in the spore divide independently of one another, and not simultaneously as in most coenocytic cells. Two chromosomes can be recognized at metaphase (Pl. VII, Fig. 32) and two at later stages proceeding to each pole.

The vegetative hyphae are narrow, less than half the diameter of those of *P. confluens*. They are richly branched and show frequent anastomoses (Pl. VI, Fig. 1). In young vegetative cells the cytoplasm is vacuolate and the nuclei (Pl. VII, Figs. 17, 20) are exceptionally clear. The granules common on the transverse walls of fungal hyphae were not observed in *A. Aurora*. No evidence was found of the occurrence of conidia or of accessory spores of any kind.

#### DEVELOPMENT OF THE SEXUAL APPARATUS.

When apothecia are about to be formed, outgrowths with dense contents appear on the mycelium (Pl. VI, Fig. 2) and develop as male or female branches. As in *Pyronema*, several pairs of sexual organs are usually concerned in the development of a single ascocarp (Text-fig. 1), the number of oogonia in a group varying, in *A. Aurora*, from one to thirty or more, with five to eleven as common numbers. Both antheridia and oogonia vary considerably in size.

The male branch terminates in a globular expansion (Pl. VI, Fig. 3) with finely granular cytoplasm and, as development proceeds, a well-marked central vacuole. The basal wall is often some distance down the stalk, so that the antheridium has the form of a drum-stick with a short, thick handle. It contains four to ten nuclei, or, in large examples, possibly one or two more. The antheridial stalk is short at first, but may undergo considerable elongation, it is often dichotomously branched (Pl. VII, Figs. 18, 19), but no case was observed in which more than one branch ended in an antheridium. Sometimes, especially when the stalk bends more or less at right angles, a balancing hypha (Pl. VI, Fig. 5, Pl. VII, Fig. 18) may be



thrown out. A similar phenomenon was observed in *Ascobolus magnificus* (12).

The stalk of the female branch is usually rather short, sometimes con-



TEXT-FIG. 1. Primordium of an ascocarp showing eight pairs of sexual organs at or soon after the stage of nuclear fusion, *a* shows a balancing hypha put out from the oogonial stalk. At *b* the trichogyne is vacuolate and the stalk of the antheridium is breaking down. At *c*, also, fertilization has taken place, the tip of the trichogyne having united with the prolongation of the antheridium down the stalk.  $\times 1,000$ .

sisting of only one cell (Pl. VI, Fig. 7, Pl. VII, Fig. 18). Balancing hyphae may be formed (Pl. VI, Fig. 10, Text-fig. 1, *a*) as on the antheridial branch. The oogonium contains dense, finely vacuolate cytoplasm, often with one or more large vacuoles (Pl. VII, Fig. 19), and with eight to twenty nuclei. In some cases it is oval, in others elongated like an Indian club (Pl. VII, Fig. 18), in others fantastically twisted or curved (Pl. VI, Figs. 4, 7-10, Pl. VI, Figs. 17, 18, 23). It is difficult in a two dimensional figure to do justice to its contortions. The proximal end is usually narrow, the distal end, in young specimens (Pl. VI, Figs. 2, 4, Pl. VII, Fig. 17), being much distended. As development proceeds, the outline is modified by the appearance of a trichogyne (Pl. VI, Fig. 4), a relatively narrow,

unicellular prolongation from the main body of the oogonium (Pl. VI, Fig. 15, Pl. VII, Fig. 18). It contains a few nuclei which later are lost. As in *Pyronema*, the trichogyne is here a secondary outgrowth, whereas, in *A. magnificus* and *Ascodesmis nigricans* it is the terminal region of the original female branch. Contact is soon made with a neighbouring antheridium (Pl. VI, Figs. 5, 6, Pl. VII, Fig. 18), and continuity is established (Pl. VII, Fig. 19) between the contents of the antheridium and trichogyne.

In due course the wall at the base of the trichogyne disappears and the male nuclei are seen (Pl. VII, Fig. 20) on their way to the oogonium, where they become associated in pairs (Pl. VII, Fig. 21) with the female nuclei. In the antheridium the nuclei are smaller and denser than those of the oogonium, but they enlarge during transit, and, when they reach the female nuclei, cannot be distinguished from them. In oogonia at this stage from eleven to thirty-one nuclei have been counted. In material that has been fixed during active development, examples are readily found of the fusion in pairs of sexual nuclei (Pl. VII, Fig. 22). Considering their small size they are strikingly clear and leave no doubt that in this fungus, as in *P. confluens* (11) and *A. magnificus* (12), normal fertilization occurs. The number of pairs of nuclei undergoing fusion in a single oogonium is from three to ten, suggesting that all the nuclei from the antheridium may reach the oogonium, and that the inactive nuclei present are surplus female nuclei. A little later, when the ascogenous hyphae are beginning to develop, the number of nuclei in the oogonium and its outgrowths ranges from eight to twenty.

TABLE I.

	Antheridia.	Virgin oogonia.	Oogonia during fusion.		Oogonia with ascogenous hyphae.
			Total no. of nuclei.	No. of pairs.	
Number of Examples counted .	19	33		28	27
Number of Nuclei . . . . .	4-10	8-20	11-31	3-10	8-20

The figures in Table I show that the number of nuclei in oogonia at the time of pairing corresponds to the numbers in antheridia and virgin oogonia taken together, and the number of pairs to the number of antheridial nuclei. At a later stage, when the development of the sporophyte has begun, the number is the same as in virgin oogonia and includes both haploid and diploid nuclei, fertilization being by this time complete.

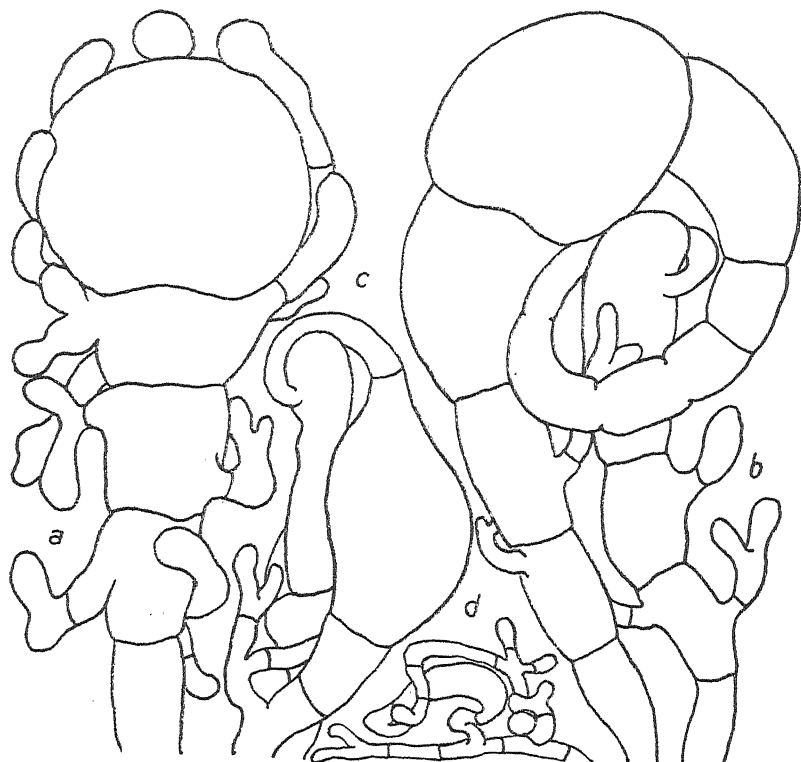
After fertilization the antheridium shows a large central vacuole (Pl. VII, Fig. 23) and a lining of dense cytoplasm, while in the trichogyne a series of vacuoles develops, giving, at first sight, the appearance of septation. In both organs deeply staining granules may be seen (Pl. VII,

Fig. 23) and, as disintegration proceeds, the whole contents (Pl. VII, Fig. 24) become densely stained. The enlargement of the oogonium at this time carries up the trichogyne to which the antheridium is firmly attached. When the antheridial stalk is long, it can accommodate itself to the resulting changes of position, where it is short the strain may become too great; it disintegrates (Pl. VI, Fig. 14) and all trace of it is ultimately lost (Pl. VI, Fig. 15). The numerous cases observed in uncut material in which the trichogyne ended in a globular expansion with no trace of independent attachment led us to doubt, during the early part of our study of this fungus, whether an antheridium was indeed lacking and the globular body oogonial in origin. Again and again what appeared to be a stalk was followed through changing foci, only to find that it merged into the curve of the trichogyne. Continued search, however, showed first one and then many cases in which the tip of the trichogyne had made contact, not with the globular region of the antheridium, but with its extension down the stalk (Pl. VI, Fig. 12, Pl. VII, Fig. 19 and Text-fig. 1, c). Lateral contact, however (Pl. VI, Figs. 11, 13, 14, Pl. VII, Fig. 24), is not uncommon, but, here also, after the male nuclei have been shed, the cells of the stalk lose their contents (Pl. VI, Fig. 13) and, even if they maintain their position, become increasingly difficult to trace.

\* DEVELOPMENT OF THE ASCOGENOUS HYPHAE.

The ascogenous hyphae are few, one to four from each oogonium, and are large in proportion to the sexual organs. Two or more nuclei pass into each (Pl. VII, Fig. 24), they divide (Pl. VII, Fig. 25), and transverse walls are formed at the first or second mitosis. The result of this septation, as in *P. confluent* (11), and *A. magnificus* (12), is a series of binucleate cells (Pl. VII, Figs. 26, 27) with a distal uninucleate cell and a proximal cell containing one or three nuclei. As in the above-mentioned fungi, the distal and proximal cells appear to take no further part in development, but the binucleate cells bud out, and the two nuclei pass into the branch and there divide again. Walls are formed across the spindles, and the branch accordingly consists of a terminal and a proximal uninucleate cell with a binucleate cell between. The middle, binucleate cell of the branch so produced may grow out to form an ascus, or may itself branch once or oftener before, from a middle, binucleate cell, an ascus is at last produced. But, however often such branching may take place, the original, binucleate cell gives rise, in branching, to only one binucleate cell and, ultimately to one ascus and no more. If, then, two nuclei enter an ascogenous hypha, and walls are formed in the first mitosis, only one binucleate cell will result and the hypha will be capable of forming only one ascus, if three nuclei enter, two binucleate cells and eventually two asci will result, and,

generally, if  $n$  nuclei enter the ascogenous hypha, the number of asci will be  $n-1$ . If, however, wall formation is delayed till the second mitosis, an ascogenous hypha which receives two nuclei will give rise to three asci,



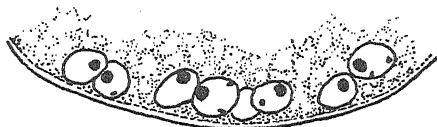
TEXT-FIG. 2. Sexual organs of fungi for comparison of size.

All are just before the stage of fertilization.  $\times 1000$ .

(a) <i>Humaria granulata</i> ,	no. of nuclei in antheridium	—	, in oogonium	600-1,400.
(b) <i>Ascobolus magnificus</i> ,	" "	"	100-400,	" 100-400.
(c) <i>Pyronema confluens</i> ,	" "	"	90-200,	" 100-200.
(d) <i>Ascophanus Aurora</i> ,	" "	"	4-10,	" 8-20.

a trinucleate hypha to five, and, generally, a hypha receiving  $n$  nuclei to  $2n-1$ . Evidently, then, the supply of asci will be greater when numerous nuclei enter the same filament than when they are distributed to several ascogenous hyphae, but, in any case, it will be conditioned by the number of diploid nuclei in the oogonium. In this connexion may be noted the curious phenomenon recorded for *Humaria rutilans* (9), *P. confluens* (11), and other fungi where the terminal cell of the ascus crozier fuses with the stalk. All the four nuclei produced by mitosis in the binucleate cell are then functional, and, if branching and division take place repeatedly and are followed by the fusion of the two uninucleate cells, a single binucleate filament may give rise to a considerable, indeed to an indefinite, number of asci.

It is worth while to emphasize that, when the nuclei are arranged in a filament in single file, as in the young, aseptate ascogenous hyphae, the only possible result of mitosis accompanied by walls across the karyo-



TEXT-FIG. 3. Male and female nuclei in the oogonium of *Pyronema confluens* just before fusion.  $\times 2,600$ . Copied from Pl. XII, fig. 13, in volume xlv. of this Journal for comparison with the nuclei shown at the same stage in Pl. VII, fig. 21.

kinetic spindles is a series of binucleate cells with a cell at either end containing a single nucleus.

The ascogenous hyphae enlarge considerably during their development; their nuclei in mitosis show four chromosomes (Pl. VII, Fig. 28), the diploid number. After the final division in the crozier two nuclei fuse, so that, as in other Ascomycetes with normal sexuality, the nucleus of the developing ascus is tetraploid. In the meiotic prophase it shows four gemini (Pl. VII, Fig. 29), and eight chromosomes are seen on the spindle in anaphase and early telophase (Pl. VII, Fig. 30), four going to each daughter nucleus. Four chromosomes again appear in the second mitosis, but, in the third (Pl. VII, Fig. 31), two only are present in metaphase, and two, the haploid number, pass to each pole.

#### DISCUSSION.

Perhaps the most outstanding character of *A. Aurora*, when compared with similar fungi, is its small size. The linear dimensions, alike of the vegetative hyphae and of the sexual apparatus, are less than half those of *P. confluens*, which is itself smaller (Text-fig. 2) than other well-known disco-mycetous forms. The number of nuclei in the sexual organs diminishes, however, with their size, the oogonium of *A. Aurora* containing only from eight to twenty in contrast to the one hundred to two hundred found in *P. confluens*. An idea of their relative size may be obtained by comparing Pl. VII, Fig. 21, with Text-fig. 3, or better, with its original, Pl. XII, Fig. 13, in volume XLV of this Journal, where the same stage is shown at the same magnification.

*A. Aurora* resembles *P. confluens* and also *P. domesticum* (14), *Ascodesmis nigricans* (2), and *Ascobolus strobilinus* (15) in the production of a compound fruit body, the ascogenous hyphae being derived from several pairs of sexual organs. A similar condition is found in *Saccobolus depauperatus* (13) and also in *Ascophanus ochraceus* (4) for which Dangeard has described a fertile branch very like that of *A. Aurora*. The oogonium is similarly twisted and bears a similar trichogyne, sometimes with a globular expansion

at the tip, like that seen in Pl. VI, Fig. 15, but Dangeard did not recognize an antheridium. His figures suggest that his species, in the light of our present knowledge of *A. Aurora*, would repay further investigation.

Like *Ascophanus ochraceus*, *Ascobolus strobilinus*, and the species of *Pyronema*, *A. Aurora* shows a distended oogonium bearing a unicellular trichogyne, which, as in *Pyronema*, is a secondary outgrowth. The oogonium of *Ascodesmis*, on the other hand, is relatively narrow and becomes septate after fertilization, while the trichogyne, like that of *A. magnificus* (12), is the distal portion of the original female branch. *A. Aurora*, however, differs from *Pyronema* in the bent or contorted form of its oogonium and also in the shape and relative size of its antheridium. The antheridia of *Pyronema* are oblong or clavate and contain about the same number of nuclei as the oogonia (Text-fig. 2), whereas the antheridia of *A. Aurora* contain only half as many nuclei as the female organs, and, apart from the frequent prolongation down the stalk, are globular or somewhat pyriform in outline.

A similar structure has been described by Dodge (5) for *Ascobolus carbonarius*, a species occurring on burnt ground and producing numerous conidia. The tip of the long, septate trichogyne comes into contact with one of these, and Dodge suggests that the apparent conidium may function as a male organ.

In the early days of the minute study of the fungi spermatia or small, globular, detached antheridia were described by several investigators as carried by external agencies to the female organs, but the technique of the period did not permit the history of their nuclei to be followed. Other observers were inclined to regard such structures as conidia, even when their cytoplasm was scant and they could not be persuaded to germinate. Interesting possibilities are opened up by the observations of Drayton (8) for *Sclerotinia trifoliorum*, of Dodge (6) for *Neurospora sitophila* and of Ames (1) for mycelia from the dwarf spores of *Pleurage anserina*, on the function of detachable bodies which they designate as spermatia or microconidia. At present the evidence is limited to the fact that, when these bodies are applied to appropriate regions of another culture, fructifications with asci develop. Ames has figured trichogynes, but does not elucidate their structure or behaviour, while, in the case of *Sclerotinia* and *Neurospora*, no information is available as to the microscopic details of the process and it is impossible to say whether the investigators are concerned with a conidium activating a mycelium of opposite complement (12), or with an antheridium making contact with a trichogyne. Should the latter prove the correct interpretation, the small, globular antheridia of *A. Aurora* will assume a special interest. It is greatly to be hoped that details of the cytology and minute anatomy of the process will before long be made available.

The investigation of *A. Aurora*, as well as of other fungi studied during the past three years, has been greatly assisted by a grant from the Department of Scientific and Industrial Research. It is a pleasure to have an opportunity of expressing our appreciation of this help. Our cordial thanks are also due to Miss Q. Hobbs, M.Sc. by whom the original material was collected in Cumberland.

#### SUMMARY.

1. *Ascophanus Aurora* is a small, orange, discomycetous fungus isolated from sheep pellets and fruiting readily in culture. The spores germinate without a period of rest, the apothecia develop as readily in darkness as in light, the fungus is monoecious and homothallic. Mitosis in the germinating spore shows two chromosomes, the haploid number.

2. The sexual organs are formed in groups, several contributing to a single fructification. The antheridium is globular, but its basal wall is often set some distance down the stalk; it contains four to ten nuclei. The oogonium is distended, like that of *Pyronema*, but is variously twisted or bent; it contains eight to twenty nuclei. The unicellular trichogyne is a secondary outgrowth and is cut off by a transverse wall. The linear dimensions both of the vegetative hyphae and of the sexual apparatus are less than half those of *P. confluens*.

3. Continuity is established between the contents of the antheridium and trichogyne, the male nuclei pass down the trichogyne to the oogonium and fuse in pairs with the female nuclei. During fusion inactive, surplus, female nuclei are also seen in the oogonium.

4. Each oogonium, after fertilization, sends out one to four ascogenous hyphae into which the fusion nuclei pass. The nuclei divide showing four chromosomes, the diploid number. After the first or second division septa are formed across the spindles and the hypha is divided into a row of cells, the distal and proximal being uninucleate, with one or more binucleate cells between them. The ascogenous hyphae give rise to asci in the usual way.

5. The nucleus of the ascus is tetraploid, four gemini are seen in the meiotic prophase and four chromosomes travel to each pole of the first spindle. Four chromosomes are also found in the second division, but two, the haploid number, are present throughout the third division.

#### POSTSCRIPT.

In the Journal of Agricultural Research for June, 1933, a paper appeared by Andrus and Harter on *Ceratostomella fimbriata* which unfortunately was seen by us too late for consideration in our discussion. The authors describe a coil of uninucleate cells which forms the female branch. We are

at a loss to understand why they regard one of its outgrowths as antheridial, since, by their own account, it is structurally identical with other branches of the oogonial stalk and plays no part in development. On equally slight grounds they suggest that, after mitosis, a nucleus from the tip of the female branch enters the second or oogonial cell and, with the oogonial nucleus, provides paired nuclei for further development. The considerable difficulties involved in the investigation of small perithecia have led them to the belief that fragmentation of the oogonium occurs. They give no details of this process or of nuclear division.

It is fortunate that Colson's thorough and convincing study of another pyrenomycetous fungus, *Neurospora tetrasperma*, is now available (see p. 211 of this volume) giving much needed chromosome counts in a form without sexual fusion and with a diploid definitive nucleus in the ascus. Her careful work on the ascus makes it doubly evident that further investigation is required before mycologists can accept Andrus and Harter's astonishing story of the transformation of a nuclear membrane into an ascus wall. It is to be hoped that a further study of *Ceratostomella* will be made with appropriate fixing fluids.

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## EXPLANATION OF PLATES VI AND VII.

Illustrating Professor Dame H. C. I. Gwynne-Vaughan and Mrs. H. S. Williamson's paper on 'The Cytology and Development of *Ascophanus Aurora*'.

### PLATE VI.

(All the figures in this Plate are from uncut material mounted undisturbed in glycerine jelly.)

- Fig. 1. Young hyphae showing lateral fusions.  $\times 1,600$ .
- Fig. 2. Hypha with developing sexual branches. The dichotomously branched filament half way down will probably give rise to an antheridium, the others to oogonia.  $\times 1,600$ .
- Fig. 3. A male branch terminating in an antheridium.  $\times 1,600$ .
- Fig. 4. Two female branches with developing oogonia, from one of which a trichogyne is being put out.  $\times 1,600$ .
- Fig. 5. Young oogonium with trichogyne and male branch showing antheridium and balancing hypha. As the antheridium is hidden behind the oogonium the male branch has been redrawn separately in a different focus.  $\times 1,600$ .
- Fig. 6. Pair of sexual branches just before the union of the trichogyne and antheridium. The narrow, proximal part of the oogonium is doubled back on itself.  $\times 1,600$ .
- Fig. 7. A twisted oogonium.  $\times 1,600$ .
- Fig. 8. The same oogonium seen in a different focus, so that the stalk is partly hidden and the tip of the trichogyne revealed.  $\times 1,600$ .
- Fig. 9. A spirally coiled oogonium.  $\times 1,600$ .
- Fig. 10. A twisted oogonium and a balancing hypha from the stalk.  $\times 1,600$ .
- Fig. 11. Pair of sexual branches with antheridium and trichogyne in continuity.  $\times 1,600$ .
- Fig. 12. Pair of sexual branches, the trichogyne has fused with the region of the antheridium which is extended down the stalk.  $\times 1,600$ .
- Fig. 13. Antheridium and oogonium after fertilization, the antheridium is nearly empty and the trichogyne vacuolate.  $\times 1,600$ .
- Fig. 14. Antheridium and oogonium after fertilization, the stalk of the antheridium is disintegrating.  $\times 1,600$ .
- Fig. 15. A twisted oogonium after fertilization, the antheridium is still attached to the trichogyne, but its stalk has disappeared.  $\times 1,600$ .

### PLATE VII.

(All the figures in this plate are from sections.)

- Fig. 16. A very young oogonium, with central vacuoles and large nuclei.  $\times 1,600$ .
- Fig. 17. A rather older group, showing three oval antheridia and an oogonium before the development of the trichogyne.  $\times 1,600$ .
- Fig. 18. Still older group with two antheridia and three oogonia. Two of the oogonia show trichogynes, the third is partly cut away. On the left the trichogyne has made contact with an antheridium from the long stalk of which a balancing hypha has been thrown out.  $\times 1,600$ .
- Fig. 19. Group of three oogonia, two of them are partly cut away. The middle oogonium is almost entire and the contents of its trichogyne are in continuity with those of the antheridium to which it is attached. Continuity is also shown between the contents of the downwardly directed trichogyne of the right hand oogonium and the attached antheridium.  $\times 1,600$ .
- Fig. 20. Section through an antheridium and oogonium showing passage of male nuclei from the former to the latter.  $\times 2,600$ .

Fig. 21. Pairing of nuclei in the oogonium.  $\times 2,600$ .

Fig. 22. Fusion of nuclei in the oogonium. Six fusing pairs may be seen and six inactive nuclei.  $\times 2,600$ .

Fig. 23. Two oogonia after fertilization. The trichogynes are vacuolate and they and the attached antheridia show deeply staining granules.  $\times 1,600$ .

Fig. 24. Two oogonia with newly formed ascogenous hyphae. The contents of the trichogynes and antheridia are disintegrating and deeply stained.  $\times 1,600$ .

Fig. 25. An oogonium and ascogenous hypha with nuclei in metaphase or early anaphase. The vacuoles in the ascogenous hypha as well as the number of dividing nuclei suggest that this may be the second mitosis after fertilization. In the oogonium a couple of inactive nuclei may be seen, presumably surplus female nuclei.  $\times 2,600$ .

Fig. 26. Oogonium and septate ascogenous hypha with three binucleate cells and a distal and proximal cell containing one nucleus each.  $\times 2,600$ .

Fig. 27. Ascogenous hypha with a terminal, uninucleate cell, a middle, binucleate cell and a basal cell containing three nuclei, the lowest wall having failed to form.  $\times 2,600$ .

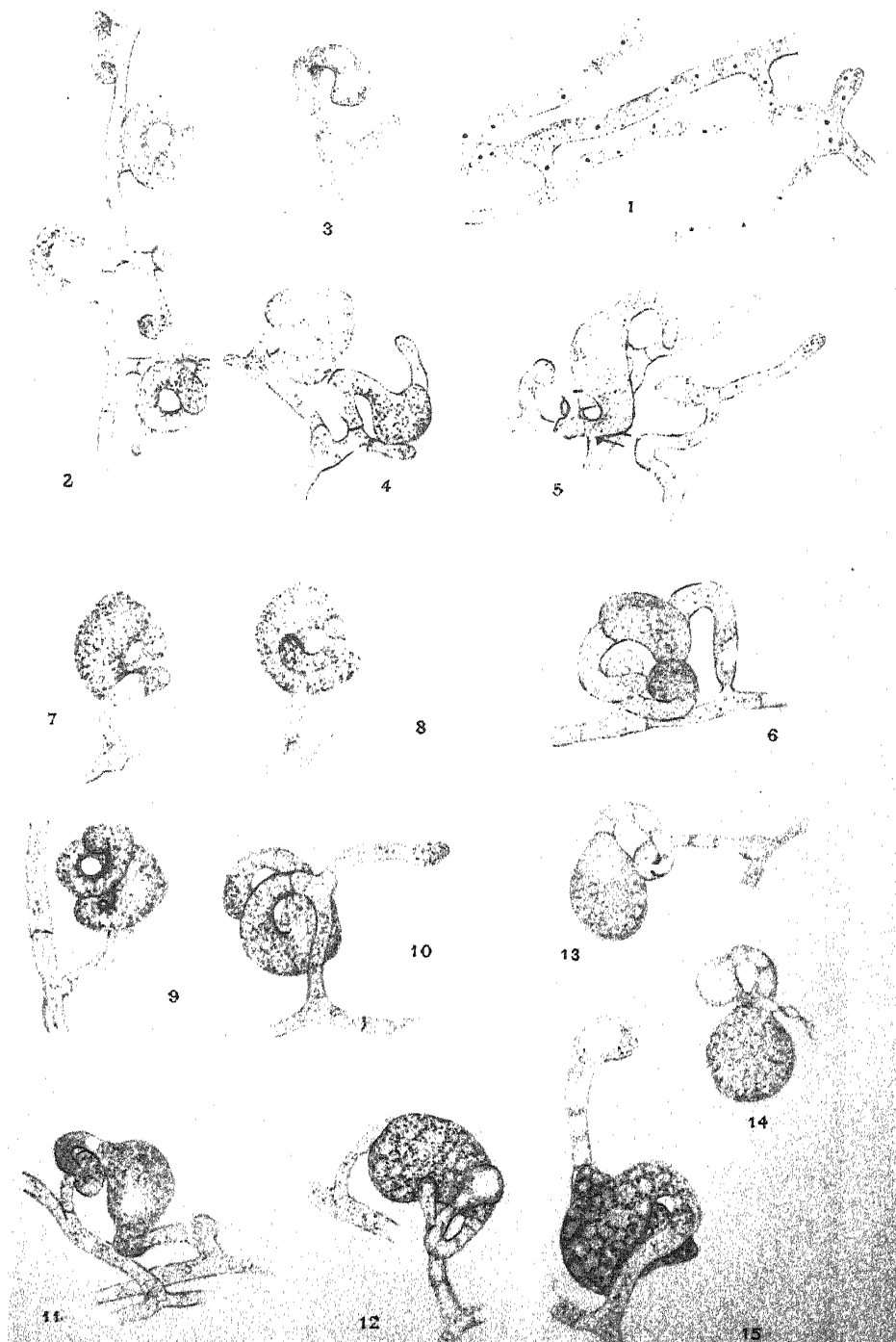
Fig. 28. Early anaphase showing four chromosomes going to each pole in the nuclei of an ascogenous hypha. The large size suggests that this an ascus crozier, the tip being cut away.  $\times 2,600$ .

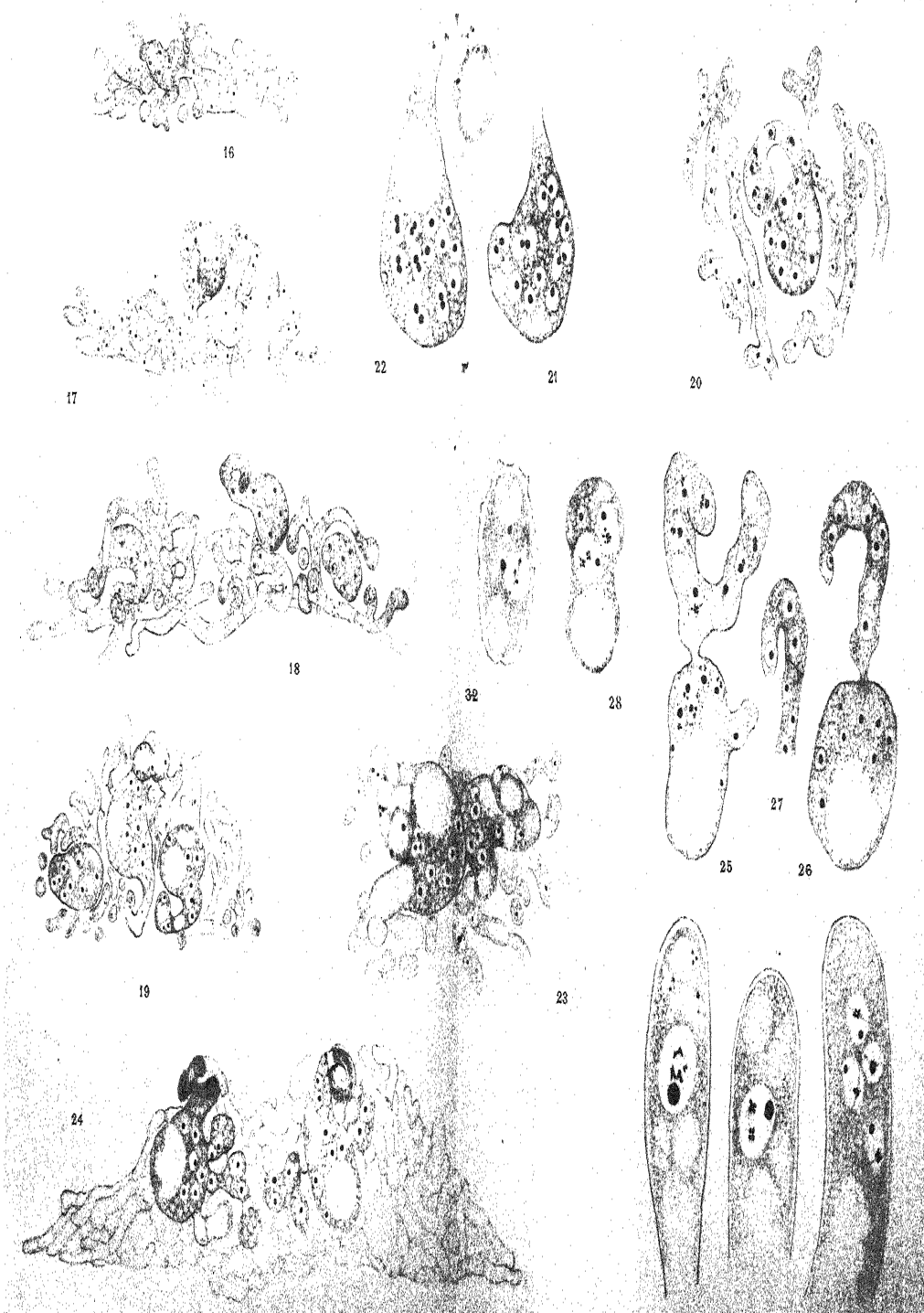
Fig. 29. Late meiotic prophase in the definitive nucleus of the ascus showing four gemini.  $\times 2,600$ .

Fig. 30. Telophase in the definitive nucleus of the ascus with four chromosomes at each pole.  $\times 2,600$ .

Fig. 31. Third division in the ascus. The two lowest nuclei are in metaphase, and each shows two chromosomes on the spindle, in the uppermost nucleus the daughter chromosomes have just separated and two are moving towards each pole.  $\times 2,600$ .

Fig. 32. Germinating spore with one of the nuclei in metaphase showing two chromosomes.  $\times 2,600$ .







## Chemical Studies in the Physiology of Apples.

### XIV. A Method of Estimating Chemical Change and Rate of Respiration in Stored Apples.

BY

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With three Figures in the Text.

THE present investigation embodies an attempt to study the rate and the character of metabolic change in the senescent phase of the apple, i.e. after picking. This phase is prolonged and passes through various stages at a slow rate. Previous investigators have worked either on chemical changes during storage or on the respiratory activity of the apple, but it is obviously desirable that these two aspects of the respiration problem should be combined.

The present paper deals with a method of observing the losses of various chemical constituents and the accompanying rate of carbon dioxide output in halves of the same apple. Some interesting correlations have thus been brought to light, but the method evidently needs improvement before it can be applied with success to individual apples or very small samples. It can, however, be used to compare samples of far smaller size than those composed of whole apples, and on this account it has seemed useful to give some detailed account of the series of experiments undertaken and of the results obtained.

It is difficult to draw conclusions from the earlier investigations of chemical change in apples since the difficulty of obtaining comparable samples and the necessity for a statistical measure of the sampling error involved was not appreciated. A number of later analyses have been published, many of them in the series of papers published under the general heading shown above; these include a number of bibliographies which deal fairly completely with the chemical analysis of the apple and make it unnecessary to recapitulate work which has been carried out in this field.

The respiration of apples has been studied in recent years by F. F. Blackman (5), Kidd and West (14), and Thomas (17, 18). Blackman, after studying the course of respiration of 21 apples in air and in different mixtures of gas, put forward a scheme of respiration and suggested that it should be possible to reach a final decision upon the problems involved by combining carbohydrate analyses with direct respiration estimations. It is hoped that the present paper may contribute to this end.

#### EXPERIMENTAL.

Worcester Pearmain and Bramley Seedling apples, varieties markedly different both in their acid content, and in the character of their skins were selected as a material for the investigation. The apples, immediately after picking, were wrapped in oiled paper and stored at 1° C., and were taken out at intervals for the experiments described below.

The investigation of Worcester Pearmain and Bramley's Seedling apples was begun in the months of December and March, 1930, respectively. These varieties could not be analysed simultaneously owing to the limited number of respiratory chambers and the work involved in collecting the respiration and chemical data. The two varieties may therefore have been at somewhat different stages of senescence.

#### METHODS OF ANALYSIS.

One half of each apple was peeled, cored, and the pulp cut finely by hand, and carefully mixed. This was used for the chemical analyses which consisted of the following estimations which were carried out by methods already described (1, 2, 3, 4, 10):

1. Dry weight.
2. Sugars: Total sugar.  
Reducing sugar.  
Sucrose by difference.  
Fructose.  
Glucose.
3. Acid.
4. Total nitrogen content.
5. Alcohol-insoluble residue.

Dry weight was determined on 10 grammes of apple and the dried material was afterwards used for the determination of total nitrogen by a micro-Kjeldahl estimation. For the determination of sugars and acids quantities from 15 to 20 grammes were extracted with alcohol. The alcohol was evapo-

rated off and the solution was made up to 200 c.c. with water. Half this quantity was nearly neutralized, cleared with basic lead acetate and delead with sodium phosphate (10); toluene was then added and the sugars were estimated in this solution which kept unchanged for months. Acids were estimated in the uncleared portion of the solution immediately after its dilution. A yellow colour of varying intensity appeared in the cleared solutions on standing.

It is to be observed that in the results published by Evans (8), fructose and glucose values do not add up to the amounts of reducing sugar estimated. This is due to the use of the values given by Lane and Eynon for the reduction of Fehling's solution by invert sugar. Since the fructose-glucose ratio in apples is usually at least 3:1 a small error is introduced by this procedure. To reduce this error a graph was prepared giving the values for solutions containing fructose and glucose in this ratio. The error due to variation of the ratio will then be small. The sums of the values for fructose and glucose have therefore been taken as the correct value for reducing sugar and these are given in the tables. The amounts of total sugar estimated have been corrected proportionally.

#### RESPIRATION.

*Description of apparatus.* The respiratory activity of half-apples was determined at 12° C. The apparatus used consisted of twenty-one glass specimen jars fitted with ground glass stoppers and of about one litre capacity (R, Fig. 1). These were used as respiration chambers. Two holes were bored in the jars, one for the inlet and the other for the outlet of the air current. The jars were placed inside a chamber maintained at a temperature of 12° C. The air in the chambers was maintained at a constant humidity of about 80 per cent.

A diagrammatic sketch of the apparatus is given in Fig. 1. The air entered the apparatus through two gas washing bottles W, containing 40 per cent. sodium hydroxide solution and through two calcium chloride towers C, before passing through the soda lime tower T. The dry air then entered a coil of 'compo' tubing D placed in the chamber to ensure constant temperature. After purification the humidity was regulated by passing the air through washing bottles  $b_1$ , containing distilled water, and  $b_2$ , containing saturated sodium chloride solution with excess of salt. The humid air then entered a bubbler  $b_3$ , which acted as a trap for moisture condensing in the tube leading to the system of parallel brass tubes connected with the respiratory chambers R, and thence to a series of glass tubes which are connected with the absorption bubblers B (consisting of a cylindrical vessel containing a loose glass spiral resting on the mouth of the inlet tube O and in which the carbon dioxide from the respiration



chambers was absorbed) by pieces of pressure tubing having a capillary tube ( $r$ ) between them. This device combined with a screw clip  $S_1$ , as shown in the diagram, was found to be very satisfactory for regulating the

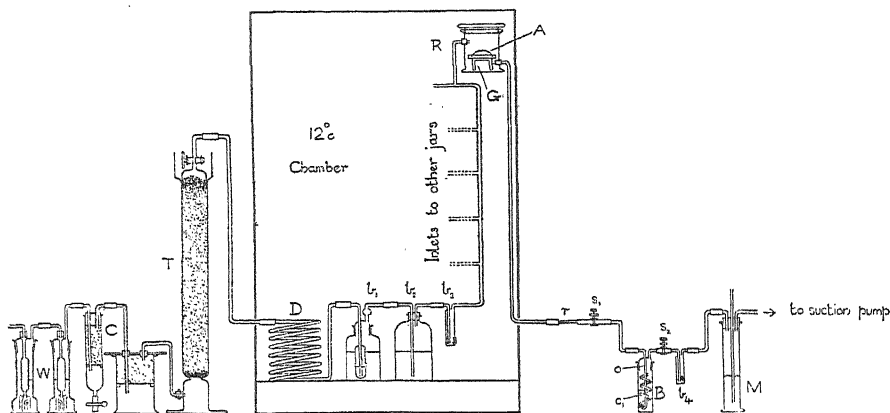


FIG. 1. Diagrammatic sketch of the apparatus used for the respiration of half-apples.

passage of air current through the apparatus. The screw clip  $S_2$  was used for final adjustment of the air current.

The air was finally drawn through a small bubbler  $b_4$  containing  $N/10$   $Ba(OH)_2$  solution to test the complete absorption of carbon dioxide in bubbler B. M represents the regulator attached to the pump.

40 to 50 c.c. of  $N/2$  sodium hydroxide were used as the absorbent liquid in each bubbler. This consisted of a sodium hydroxide solution prepared from a saturated solution which had been kept for two or three weeks, to allow the carbonate to settle.  $N/2$  soda thus obtained was found to be free from carbonate.

After absorption, the contents of the bubbler were transferred to a 250 c.c. graduated flask and rinsed free of soda with carbon dioxide free water. About 5 c.c.  $N/1$   $BaCl_2$  were added to precipitate the carbonate, the solution was then made up to 250 c.c. with  $CO_2$  free water and allowed to settle overnight. From this solution two 100 c.c. portions of the supernatant liquid were siphoned off. To avoid increase in the bulk of the titration liquid 50 c.c. of  $N/10$  hydrochloric were added from an automatic pipette and the end point was determined by  $N/20$  hydrochloric acid, using two drops of 0.2 per cent. phenolphthalein as indicator. The duplicate titration agreed within one to two drops of  $N/20$  hydrochloric acid.

*Technique of carbon dioxide measurements.* In all the apparatus where rubber tube was used care was taken to have the ends of the glass tubes come in contact at the joints, because of the well-known fact that rubber absorbs carbon dioxide selectively.

Each half-apple (A, Fig. 1) was embedded in wax in a Petri dish which rested on a glass triangle G in a respiration chamber during the experiment. The ground stoppers of the respiration chambers, well lubricated with vaseline, readily gave an air-tight joint, being forced well into place by the negative pressure used in the experiment. Before starting each experiment, the whole of the apparatus was tested during three days and the joints were always found air-tight. Air was passed through the chambers containing the half-apples for twenty-four hours, after which absorptions of carbon dioxide were begun. The air current was drawn through the chambers (21 in number) at a rate of 25 or 30 litres per hour. An absorption bubbler was connected to an empty control respiration chamber during each period of absorption, and the carbon dioxide absorbed was calculated from the difference between the control and the experimental titration value.

#### TECHNIQUE OF THE HALF-APPLE METHOD.

*Method of cutting and embedding in wax.* Preliminary experiments concerning the half-apples were carried out by Dr. Janet Brown in this laboratory. She showed that the difference both in acidity and specific gravity between the halves is very small. It is least when the apple is cut longitudinally, i.e. through calyx and stalk, but even when the apple is cut transversely, the mean difference between halves is very much less than the difference between the means of small samples. Evans (8) used this method for storage experiments on a set of Bramley's Seedling apples and found that it worked satisfactorily at 2° C.

The whole technique of the method has since been improved. As the experiments described later were carried out at high temperatures of 12° and 15° C., special care was exercised in cutting and waxing the halves under as sterile conditions as possible to avoid fungal attacks at the cut surface.

The apple was weighed, wiped with cotton wool dipped in alcohol and cut longitudinally through calyx and stalk with a sharp knife dipped in alcohol and flamed. As far as possible the halves were so cut as to give equal distribution of the area of deeper skin colour between the two. Paraffin wax, of melting point 50° C. was heated at 120° C. in a tin, cooled and kept melted in a water bath for one hour before use, and then poured while hot into Petri dishes. One half of the apple was embedded in the wax immediately after cutting, its cut surface being kept downwards during manipulation to avoid contamination from the atmosphere. The other half was weighed in a covered glass dish, a separate dish being used for each half. In the earlier stage of the investigation a common weighing dish was used, but the chance of fungal attack was much reduced by weighing each half in a separate sterilized dish. The half was embedded

to such a depth as to cover the stalk and calyx completely in paraffin. The weight of the embedded half-apple was determined by the difference between the weight of the whole apple and the weight of the other half.

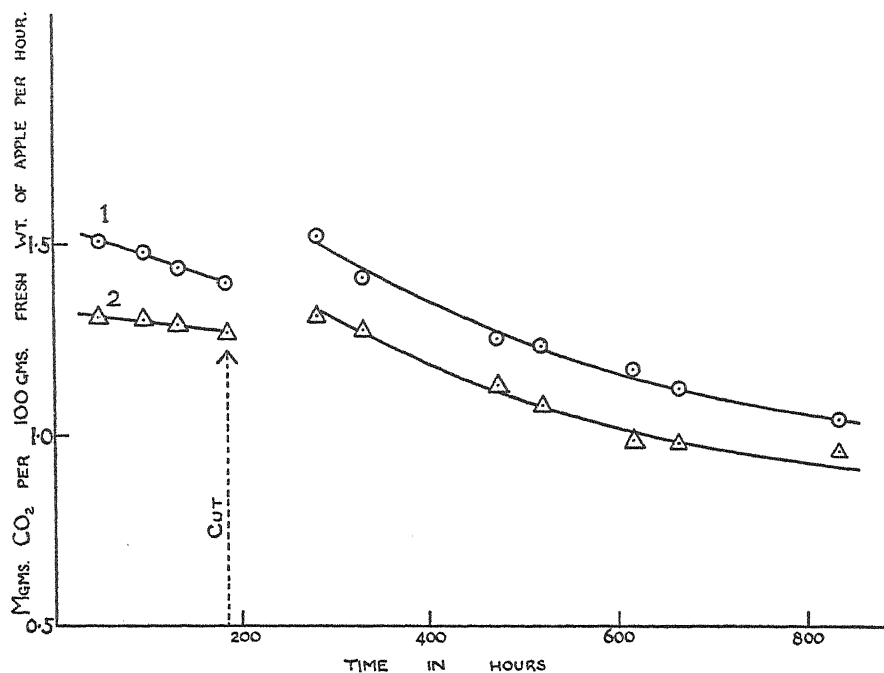


FIG. 2. Curves showing the respiration at 12°C. of two Bramley's Seedling apples before and after cutting.

#### *Wound Effects and the Respiration of Half-apples.*

Bohm (7) called attention to a considerable increase in the amount of carbon dioxide produced by potatoes by cutting in various ways. Stich (16) continued researches in this connexion and published a more extended account of this phenomenon in the same and other plants. Richards (15) investigated this phenomenon in various plant tissues and stated that this increased activity of respiration after reaching, usually within two days, a maximum, falls gradually, as the wound heals over, to a normal or to an almost normal rate. Wound effects on the respiratory activity in apples have been studied by Magness and others (11) and a temporary increase in the respiratory activity due to different forms of injury has been observed. It was therefore found necessary to determine the length of period over which the temporary increase in respiration lasted under the conditions of the present investigation, so that errors in the carbon dioxide measurements due to the temporary increase of respiratory intensity could be avoided by delaying the observations till the normal rate was resumed.

The curves of Fig. 2 give the course of respiration at 12° C. of two Bramley's Seedling apples before and after cutting. The first part of the curves representing the whole apples No. 1 and 2 indicates their respiratory activity over a period of eight days (192 hours). It is seen that the respiration of these apples gradually declines. After eight days, both the apples were cut and embedded in wax, and their respiratory activities were again determined. The respiration curves of the cut apples are shown by the second half of each curve. It is evident that after cutting the respiratory activity of the apples has markedly increased, but when examined after a period of about seven days the curve is seen to be falling.

In Table I is given a detailed comparison of the chemical composition of the companion halves of apples of the Worcester Pearmain variety from Canterbury. These experiments have been followed by a second set of analyses (Table II) of Worcester Pearmain apples from Malvern, i.e. belonging to another population. In these apples the carbon dioxide output of the corresponding halves was measured. The rates of respiration of the various pairs of halves are shown graphically in Fig. 3.

TABLE I.

*Comparison of the Composition of Halves of Single Apples immediately after Cutting. All Data calculated on 100 grm. Fresh Weight.*

Worcester Pearmain (Canterbury).						
Apple.	Dry weight.	Total sugar.	Reducing sugar.	Sucrose.	Fructose.	Glucose.
1 A	16.43	12.40	8.93	3.47	7.12	1.81
1 B	15.36	11.90	8.60	3.30	6.88	1.72
2 A	15.24	11.82	7.88	3.94	6.24	1.64
2 B	15.91	12.33	7.91	4.42	6.39	1.52
3 A	16.84	13.46	8.68	4.78	7.08	1.60
3 B	17.40	13.86	9.35	4.51	7.68	1.67
4 A	16.40	12.07	8.53	3.54	6.36	2.17
4 B	15.83	11.42	7.93	3.49	6.20	1.73
5 A	14.72	11.20	8.29	2.91	6.68	1.61
5 B	14.35	10.99	8.18	2.81	6.60	1.58
6 A	15.07	11.40	8.31	3.09	6.68	1.63
6 B	15.25	11.84	8.45	3.39	6.76	1.69
7 A	15.20	11.24	8.78	2.46	6.44	2.34
7 B	15.55	11.41	8.52	2.89	6.48	2.04

It was found by examination of the half-apples that Nos. IVA, VA, VIII A, and IX B were attacked by fungi, and the graph shows that the respiratory activity of these halves increased. This observation is in accordance with those of other workers. It is well known that carbon dioxide output of a diseased apple rises rapidly with the progress of the fungal invasion. No appreciable variation in the respiratory activity of the

halves of apples Nos. I, II, VI, and X is noticeable. The halves of apples Nos. III, IV (A attacked by fungus about the sixth day of the experiment),

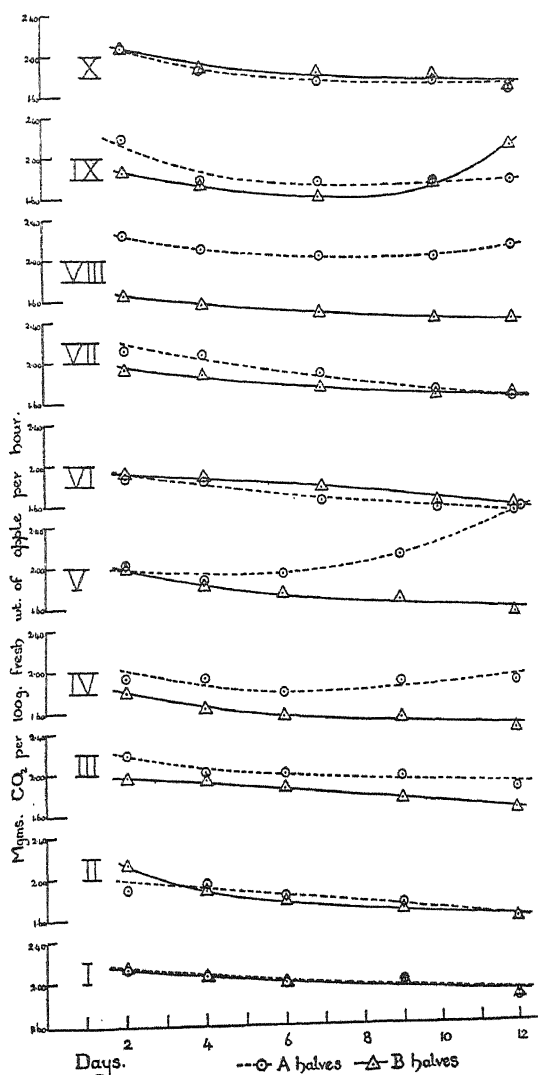


FIG. 3. Time-respiration curves of corresponding halves of ten apples over a period of twelve days at 12°C. A and B halves shown separately.

VII, IX (B attacked by fungus about the eighth day of the experiment), show slight differences in their initial respiratory rates. In A of apple VIII, fungal attack was observed at the end of the experiment. It is probable that fungal attack had begun in the core at the start.

TABLE II.

*Half-apples. Analytical Data and Total Respiratory Loss during 12 Days at 12°C. (gram. per 100 gram. Fresh Weight).*

Worcester Pearmain (Malvern). November, 1930.

Apple.	Nitrogen.	Dry weight.	Acid as malic.	Total sugar.	Reducing sugar.	Sucrose.	Fructose.	Glucose.	Carbon dioxide loss
1 A	0.0279	15.76	0.184	11.63	9.94	1.69	7.60	2.34	0.559
1 B	0.0317	15.17	0.179	11.32	9.64	1.68	7.09	2.55	0.562
2 A	0.0252	15.06	0.238	11.44	9.18	2.26	7.13	2.05	0.501
2 B	0.0251	14.58	0.238	11.69	9.41	2.28	7.40	2.01	0.502
6 A	0.0226	14.80	0.245	11.23	9.95	1.28	6.97	2.98	0.476
6 B	0.0285	15.46	0.240	11.68	10.39	1.29	7.04	3.35	0.503
7 A	0.0279	13.27	0.238	9.85	8.74	1.11	6.88	1.86	0.528
7 B	0.0285	14.21	0.233	10.78	9.26	1.52	7.20	2.06	0.504
10 A	0.0237	17.38	0.364	12.98	10.47	2.51	7.77	2.70	0.511
10 B	0.0240	16.23	0.358	12.45	10.27	2.18	7.57	2.70	0.521

*Similarity of Halves of Individual Apples.*

*Chemical composition.* The results of the analysis of Worcester Pearmain apples from Canterbury and Malvern given in Tables I and II show that the individual apples of the same variety exhibit considerable differences in their chemical composition, but that the variation between the halves of the same apple is a little smaller.

TABLE III.

*A Comparison of the Differences of Sugar Content between Halves of the same Apple and the Deviations from the Mean of the Sugar Contents of the same Apples.*

Worcester Pearmain. (Canterbury).		Worcester Pearmain. (Malvern).	
Difference of halves.	Deviation from mean.	Difference of halves.	Deviation from mean.
0.50	0.20	0.31	0.03
0.51	0.13	0.25	0.06
0.40	1.17	0.45	0.05
0.65	0.21	0.93	1.19
0.21	0.85	0.53	1.21
0.44	0.33		
0.17	0.63		
Mean	0.411	0.494	0.508

Some idea of the variation between different halves and that between individual apples may be obtained from Table III in which the differences

of the sugar content of two halves is compared with the deviation of each apple, *taken as a whole*, from the mean of the sample. It is noticeable that in the second set the mean of the differences is almost exactly equal to that of the deviations. In the first set the differences between halves are smaller, but not so small as was to be expected from other experiments which have been carried out upon half-apples. This is probably to be attributed to the method of cutting through the middle of the region of maximum coloration. It suggests that the orientation of the apple on the tree plays a part in determining the distribution of sugar, and this hypothesis is supported by the fact that the range of values obtained for differences is very small as compared with those given by deviations. It has not been possible to test other methods of cutting Worcester Pearmain during the present series of experiments, but the close agreement of the individual differences between the halves supports the assumption of some definite cause of difference and also justifies the use of the mean difference, where positive and negative differences are fairly equally divided as representative of the sugar content of the sample. These conclusions must, however, be regarded as tentative, and they await further confirmation.

#### *'Grouping' of the Half-apples.*

Since, in the experiments described in the sequel, the mean respiratory values of one *set* of halves was to be compared with the mean value of the second *set* of halves after the lapse of the time allowed for respiration, the slight variations in the respiratory activity of the halves of an apple observed above suggested that the two sets of half-apples employed for an experiment should be grouped according to their initial respiratory rate, each set being arranged so that it contained an equal number of higher and lower respiring halves. This arrangement broke down owing to fungal attack, but it is not to be recommended since, as will appear from the tables, rate of respiration and total sugar show little or no correlation.

#### *Comparison of Chemical Composition, Chemical Change and Rate of Respiration.*

Nine<sup>1</sup> Worcester Pearmain apples and nine Bramley's Seedlings from orchards near Burwell were cut into halves and embedded in wax; all the halves were kept at 12° C. for about two weeks to resume their normal respiration after wounding. One half of each apple was then analysed (Tables IV and V) and the carbon dioxide output (Tables VI and VII) of the other half of each apple in the two varieties was determined separately

<sup>1</sup> Analysis of only seven halves in each sample could be completed owing to fungal attack, &c.

over a period of six weeks at 12° C. At the end of this period the second halves were analysed individually (Tables IV and V).

At the same time as the above experiment with Bramley's Seedling (Burwell) apples was carried out, a *mixed sample* of one set of halves of the same variety from Canterbury was analysed (Table VIII). The respiratory activity of the corresponding halves was then determined, and these were analysed as a mixed sample after the respiration was concluded.

*Respiratory Activity and the Chemical Composition of the  
Substrate in the Apple.*

From the data of Tables IV–VIII respiration values were plotted against the concentration of different chemical constituents of the same halves, but no definite correlation could be obtained between the respiration and the chemical composition of the tissue, except that high nitrogen content seemed to be associated with high respiration rate. Kidd, West, and Archbold (14, year 1924) showed that the respiratory activity of a series of individual Bramley's Seedling apples was in general accompanied by a high nitrogen content. Thus the results presented confirm the observations of these workers.

*Comparison of Chemical and Respiratory Losses.*

The analytical results of Tables IV and V show that no correlation between the chemical losses and the corresponding respiration can be established for individual apples by the above described methods; moreover the variation in the amount of total sugar oxidized in each case is not of the same order of magnitude as the variation in the amount of carbon dioxide produced. These discrepancies may be largely attributed to differences in the chemical composition of the halves of each apple at the time of bisection since the positive or negative difference between the halves is included in the apparent loss of sugar and acid, and this is compared with the carbon dioxide output of a single half. Discrepancies of this nature should cancel out in the mean if the sample of apples is sufficiently large, but in the present instance it cannot be assumed that the errors vanish in the mean. The equivalence of the mean losses of sugar and acid and of carbon dioxide is therefore compared in the first instance by calculating the carbon content of each quantity as estimated. Table IX gives the mean losses of total sugar and acid taken from Tables IV and V together with the corresponding losses of carbon dioxide; similar data are also taken from Table VIII. All values are expressed as percentages of the fresh weight of the apple and in terms of carbon content. From the data of Table X the significance of the results can be tested.



TABLE IV.  
*Chemical and Respiratory Data of Worcester Pearmain (Burwell).*

A. First analysis of half-apples calculated as gm. per 100 gm. fresh weight of apple.  
B. Second analysis, after six weeks at 12°C. calculated as gm. per 100 gm. original fresh weight of apple.

Apple.	Loss of fresh weight. %.	Nitrogen.	Acid as malic.	Dry weight.	Alcohol insol. residue.	Total sugar.	Reducing sugars.	Sucrose.	Fructose.	Glucose.	CO <sub>2</sub> output.
1 A		0.0259	0.142	14.85	1.94	11.65	10.37	1.27	7.55	2.82	
1 B	3.12	0.0269	0.113	13.46	1.89	10.16	9.91	0.25	6.71	3.20	1.184
Difference		+0.0010	-0.029	-1.39	-0.05	-1.49	-0.46	-1.02	-0.84	+0.38	
2 A		0.0293	0.135	15.03	2.09	11.61	10.50	1.11	7.73	2.77	
2 B	3.76	0.0318	0.104	14.45	1.89	11.10	10.54	0.56	8.09	2.45	1.284
Difference		+0.0025	-0.031	-0.58	-0.20	-0.51	+0.04	-0.55	+0.36	-0.32	
3 A		0.0315	0.135	15.86	2.11	12.24	11.22	1.02	8.50	2.72	
3 B	3.46	0.0303	0.144	15.34	2.07	11.74	11.07	0.67	7.92	3.15	1.404
Difference		-0.0012	+0.009	-0.52	-0.04	-0.50	-0.15	-0.35	-0.58	+0.43	
4 A		0.0336	0.135	15.09	2.00	11.54	10.67	0.87	7.49	3.18	
4 B	3.91	0.0313	0.099	14.08	1.95	50.38	10.35	0.03	6.75	3.60	1.392
Difference		-0.0023	-0.036	-1.01	-0.05	-1.16	-0.32	-0.84	-0.74	+0.42	
5 A		0.0233	0.190	14.96	2.01	11.61	10.71	0.90	8.25	2.46	
5 B	3.07	0.0248	0.192	13.30	1.89	9.77	9.49	0.28	7.10	2.39	1.140
Difference		+0.0015	+0.002	-1.66	-0.12	-1.84	-1.22	-0.62	-1.15	-0.07	
6 A		0.0221	0.162	14.79	2.02	11.58	10.79	0.79	8.70	2.09	
6 B	3.61	0.0227	0.104	14.68	1.99	11.36	11.16	0.20	9.13	2.03	1.171
Difference		+0.0006	-0.058	-0.11	-0.03	-0.22	+0.37	-0.59	+0.43	-0.06	
7 A		0.0242	0.224	18.65	2.46	14.33	12.16	2.17	9.00	3.16	
7 B	5.13	0.0227	0.179	17.85	2.36	13.06	12.66	1.00	10.06	2.60	1.309
Difference		-0.0015	-0.045	-0.80	-0.10	-0.67	+0.50	-1.17	+1.06	-0.56	
Mean loss and gain of 7 apples (% on fresh weight).	3.72	+0.0014	-0.027	-0.81	-0.08	-0.913	-0.18	-0.73	-0.21	+0.03	1.269

TABLE V.  
*Chemical and Respiratory Data of Bramley's Seedlings (Burwell).*

A. First analysis of half-apples calculated as grm. per 100 grm. fresh weight of apple.  
B. Second analysis, after 6 weeks at 12°C., calculated as grm. per 100 grm. original fresh weight of apple.

Apple.	Loss of fresh weight, %.	Nitrogen.	Acid as malic.	Dry weight.	Alcohol insol. residue.	Total sugar.	Reducing sugars.	Sucrose.	Fructose.	Glucose.	CO <sub>2</sub> output.
1 A		0.0172	0.738	14.14	2.04	9.93	8.05	1.88	5.95	2.10	
1 B	1.39	0.0180	0.603	13.23	1.83	9.33	8.26	1.07	6.07	2.19	0.701
Difference		+0.0008	-0.135	-0.91	-0.21	-0.60	+0.21	-0.81	+0.12	+0.09	
2 A		0.0174	0.706	13.01	2.04	9.24	7.62	1.62	5.65	1.97	
2 B	1.86	0.0177	0.584	12.38	1.84	8.67	7.65	1.02	5.78	1.87	0.789
Difference		+0.0003	-0.122	-0.63	-0.20	-0.57	+0.03	-0.60	+0.13	-0.10	
3 A		0.0211	0.711	12.13	1.67	8.91	7.39	1.52	5.29	2.10	
3 B	1.15	0.0228	0.575	10.82	1.61	7.66	7.04	0.62	4.99	2.05	0.773
Difference		+0.0017	-0.136	-1.31	-0.06	-1.25	-0.35	-0.90	-0.30	-0.05	
4 A		0.0187	0.784	12.30	1.82	8.63	7.18	1.45	5.25	1.93	
4 B	1.27	0.0172	0.621	11.25	1.72	7.68	6.98	0.70	4.48	2.50	0.789
Difference		-0.0015	-0.163	-1.05	-0.10	-0.95	-0.20	-0.75	-0.77	+0.57	
5 A		0.0196	0.704	12.37	1.87	8.76	7.30	1.46	5.30	2.00	
5 B	1.72	0.0184	0.562	11.08	1.72	7.50	6.64	0.86	5.06	1.58	0.878
Difference		-0.0012	-0.142	-1.29	-0.15	-1.26	-0.66	-0.60	-0.24	-0.42	
6 A		0.0253	0.753	12.99	1.82	9.25	7.89	1.36	5.35	2.54	
6 B	1.89	0.0269	0.604	11.45	1.69	7.98	7.29	0.69	4.52	2.77	0.898
Difference		+0.0016	-0.149	-1.54	-0.13	-1.27	-0.60	-0.67	-0.83	+0.23	
7 A		0.0187	0.704	11.67	1.68	8.50	7.10	1.39	4.99	2.11	
7 B	1.75	0.0196	0.581	10.67	1.63	7.54	6.91	0.63	4.81	2.10	0.778
Difference		+0.0009	-0.123	-1.00	-0.05	-0.96	-0.19	-0.76	-0.18	-0.01	
Mean loss and gain of 7 apples (% on fresh weight).	1.57	+0.0008	-0.139	-1.10	-0.13	-0.98	-0.25	-0.73	-0.29	+0.04	0.801

TABLE VI.

*Respiratory Activity of Half-apples at 12° C. over Successive Periods of 48 to 121 Hours during Six Weeks beginning 19. 12. 30. Vertical Columns give mg. CO<sub>2</sub> per 100 grm. Fresh Weight per Hour.*

Worcester Pearmain (Burwell).

Periods. Length of period (hrs.).	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.
48	118.5		97.0	120.5	117.5	96.0	121.0	119.5	48.0	95.5
Half apple 1.	1.32	1.33	1.24	1.21	1.16	1.16	1.17	1.18	1.15	1.16
" 2.	1.37	1.36	1.36	1.25	1.37	1.26	1.26	1.30	1.28	1.28
" 3.	1.47	1.47	1.46	1.45	1.49	1.42	1.40	1.42	1.35	1.32
" 4.	1.65	1.62	1.49	1.43	1.40	1.34	1.34	1.33	1.32	1.33
" 5.	1.33	1.30	1.24	1.21	1.17	1.09	1.08	1.09	1.05	1.07
" 6.	1.30	1.29	1.28	1.25	1.13	1.17	1.12	1.15	1.13	1.13
" 7.	1.52	1.52	1.42	1.43	1.28	1.30	1.25	1.30	1.24	1.10
" 8.	1.35	1.34	1.37	1.32	1.27	1.22	1.25		Fungal attack	

TABLE VII.

*Respiratory Activity of Half-apples at 12° C. over Successive Periods of 141 to 170 Hours during Six Weeks from 28. 2. 31. Vertical Columns give mg. CO<sub>2</sub> per 100 grm. Fresh Weight per Hour.*

Bramley's Seedling (Burwell).

Periods. Length of period (hrs.).	I.	II.	III.	IV.	V.	VI.
141.5		168.0	168.0	168.5	168.0	170.0
Half apple 1.	0.82	0.78	0.74	0.69	0.65	0.61
" 2.	0.89	0.88	0.87	0.76	0.73	0.69
" 3.	0.90	0.86	0.84	0.76	0.71	0.66
" 4.	0.89	0.89	0.82	0.79	0.67	0.71
" 5.	0.97	0.94	0.90	0.87	0.88	0.80
" 6.	1.05	0.99	0.95	0.83	0.87	0.81
" 7.	0.93	0.86	0.81	0.76	0.73	0.67
" 8.	0.94	0.91	0.91	0.98		Fungal attack.



It is seen from the above results that in the Worcester Pearmain about 8 per cent. of the sugar and acid lost appears not to be accounted for as carbon dioxide, while in both sets of Bramley's Seedling the excess of sugar and acid mounts to about 50 per cent. The difference of behaviour between the samples of two varieties of apple is therefore remarkable whether it be ascribed to difference of variety or to difference in degree of senescence, but as has been already pointed out it cannot be accepted as established without further evidence on account of the small number of apples submitted to experiment. There is, however, considerable probability that the metabolism of the samples of Bramley's Seedling differs from that of the Worcester Pearmain sample. It should also be noticed that the loss of dry weight in both sets of apples agrees very closely with that of sugar and acid. It is clear, therefore, that carbon dioxide produced by the oxidation of sugar is not resynthesized in the apple to any non-volatile product.

It is customary to estimate the probability that a difference found by experiment may be due to chance variation by the use of 'Student's' method as modified by Fisher (9), and Table X has been calculated for this purpose. It shows the ratios of sugar and acid lost to carbon dioxide produced, again in terms of carbon content, for the pairs of half-apples and also the difference of these ratios from unity with the standard deviations of these mean differences. The table makes clear at a glance that the mean difference from unity of the ratios is large and statistically significant for the Bramley's Seedlings, but is small and non-significant for the Worcester Pearmain. If the variance were small this test would afford sufficient evidence that the Worcester Pearmain oxidized most of the sugar lost in respiration to carbon dioxide, and that this is not true of the Bramley's Seedlings; but the variance, especially in the Worcester set, is very large, and the close approximation to unity of the mean ratio obtained for this latter set of apples cannot therefore be regarded, without further evidence, as characteristic of the sample. Further support is, however, to be found in the fact that four out of seven ratios are less than unity. This can be accounted for by differences in the sugar content of corresponding half-apples, and it is not easy to account for the discrepancy in any other way since the apple contains little material but sugar available for respiration. The remaining three ratios which are greater than unity are presumably affected by differences of sugar content of opposite sign. On this hypothesis halves of high and low sugar content belonging to the Worcester Pearmain variety were divided as equally as possible between the sets first and last analysed, which is, of course, the distribution of maximum probability. This interpretation of the results is confirmed by the analyses shown in Tables I and II which give the sugar content of halves analysed at one time—the halves were found to exhibit differences

of the same order as those accounted for as sugar in the sample respired.<sup>1</sup>

TABLE X.

*Ratio of Sugar and Acid Lost to Carbon Dioxide Lost (in Terms of Carbon Content) and the Difference of this Ratio from Unity (see Tables IV and V).*

Apple.	Ratio.	Difference of ratio from unity.	Ratio.	Difference of ratio from unity.
	Bramley's Seedling.		Worcester Pearmain.	
1.	1.51	+0.51	1.88	+0.88
2.	1.27	+0.27	0.61	-0.39
3.	2.61	+1.61	0.51	-0.49
4.	2.04	+1.04	1.26	+0.26
5.	2.32	+1.32	2.36	+1.36
6.	2.29	+1.29	0.34	-0.66
7.	2.02	+1.02	0.80	-0.20
Mean difference with S.E.		+1.051 ± 0.1739	+0.109 ± 0.2428	

# CONCLUSION.

It must be emphasized that the experiments described are primarily a study of method and that time and circumstance did not permit a complete series of results to be obtained. The method is obviously capable of extension and improvement: large samples of half-apples should show the relationship between loss of sugar and output of carbon dioxide with considerable accuracy. It is hoped that improvements in the method may enable the correlation of these quantities to be studied in relation to the individual apple. It should thus be possible to obtain a more complete picture of the two principal aspects of the progress of senescence in the apple—those relating to chemical change and respiratory phenomena respectively—than has been hitherto afforded by experiments on whole apples.

The results obtained by experiments on small samples of Worcester Pearmain and Bramley's Seedling apples suggest a definite difference in the metabolism of these two varieties which, however, requires confirmation. There is, however, a considerable probability that in a majority of the Worcester apples investigated the sugar lost underwent a more or less complete oxidation to carbon dioxide. If this be established it will constitute an unusual and interesting phenomenon of respiration which may be characteristic of all apples in the earlier stages of senescence or may be the consequence of properties peculiar to apples of the Worcester Pearmain

<sup>1</sup> The experiment on Worcester Pearmain apples described above has again been carried out in this laboratory. Almost exactly the same value was obtained for the mean ratio and the distribution of the ratios above and below unity was similar. The variance was not so great.

type. The evidence for incomplete oxidation in the samples of Bramley's Seedling is convincing; this more ordinary type of respiration may be a consequence of the later date at which the samples of this variety were respired. The observations indicate that some products other than carbon dioxide must result from the oxidation of sugar. Alcohol and aldehyde have been detected among the products of respiration of apples in atmospheres which contain excess of carbon dioxide (17, 18), and it would seem not improbable that the waxy coating of Bramley's Seedling apples may affect the course of respiration of these apples. A recent paper by Markley and Sando (12) is of interest in this connexion. These investigators showed that the waxy coating of a number of apple varieties tended to increase during storage. In this case some part of the sugar lost must in all probability be accounted for as wax, and increase of wax with its attendant blocking of the lenticel may well play a further part, since the supply of oxygen will be curtailed and changes in the character of respiration result. These considerations are indications of a number of unsolved problems of apple metabolism, the solution of which would be greatly facilitated by a knowledge of the relation of sugar consumption to carbon dioxide production throughout the period of senescence. It is hoped that the experiments described will contribute to this end.

#### SUMMARY.

A comparison has been undertaken of the respiration losses of apples and of the changes in chemical composition of apples, the half-apple method being employed.

A detailed study of the 'half-apple method' showed that the differences in the composition of the corresponding halves is somewhat smaller than the difference of individual apples. Only a very small variation in the respiratory activity of the corresponding halves of an apple was observed.

Cutting of the apples produced an increase in rate of respiration. It was shown that the increase of rate disappeared in about seven days at 12°C. Estimations of the output of carbon dioxide were therefore not undertaken until ten to fourteen days after halving the apples.

A study of the chemical changes and the rate of respiration at 12°C. was carried out over a period of six weeks for apples of the Worcester Pearmain and Bramley's Seedling varieties obtained from the same locality. These varieties differ in the character of the skin, the rate of respiration, and the initial content of acid.

The variance in the ratio of sugar plus acid lost to carbon dioxide produced was large, especially for Worcester Pearmain, and the samples of apples were small. The large difference in the values of the mean ratio

obtained for the two varieties cannot therefore be regarded as completely established. It is probable, however, that in the Worcester set the loss of sugar plus acid was nearly equivalent to the loss of carbon dioxide, while in the Bramleys some considerable part of the chemical losses was not accounted for.

It is shown that no non-volatile products were synthesized from carbon dioxide during the respiration of the latter set of apples, but that it is probable that volatile compounds such as alcohol, aldehydes, and esters are given off.

It is suggested that the different type of respiration which appears to be characteristic of the Bramley's Seedlings may be due to the waxy coating of the skin and that accumulation of wax may account for some part of the sugar which disappears in the process of respiration. A similar lack of agreement between respiratory and chemical losses was observed in a sample of Bramley's Seedling from a second locality.

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## Studies in the Suction Pressure of Plant Cells. II.

BY

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With one Figure in the Text.

### *The measurement of suction pressure.*

AS has been shown earlier (1), no two cells in any row lying between the water-supplying tracts and the surface of the plant from which water is lost can have the same suction pressure, but there must be a gradually increasing suction pressure as we pass from cell to cell towards the surface. Therefore the removal of cells from a plant immediately brings about a change in suction pressure in these cells, and the suction pressure of any cell in an isolated fragment of tissues depends in part on its original suction pressure, in part on the suction pressure of all the cells isolated with it.

The necessity of avoiding injury (1) to any cells in obtaining material for suction pressure measurements limits the choice of material usually to the outer layers of tissue. The epidermis cannot usually be used for suction pressure measurements because the walls of the epidermis are usually not extensible to any marked extent, which makes such cells difficult or impossible of employment. In many plants, however, as has already been described (p. 720), the epidermis can be torn away from the leaf in such a way that one row of mesophyll cells, with extensible walls, remains attached to it. Such strips may be isolated from an intact plant and plunged straight into paraffin. Alternatively an organ of the plant may be isolated and, after allowing time sufficient to cause the natural water gradients in the part to disappear, a suitable strip may then be isolated.

The value of the *equilibrium suction pressure of the epidermis and the first stratum of mesophyll cells*, or that of the *equilibrium suction pressure of all the cells of the leaf* or other organ, can thus be obtained.

Paraffin has no effect on the suction pressure of isolated superficial tissues which have been employed, as far as can be determined: there is no change in suction pressure with length of immersion from the first three minutes up to eight or ten hours, although the possibility of some instantaneous effect of immersion in paraffin remains. The observations necessary for the measurement of any cell necessitate the preparation

remaining at least three minutes in paraffin, so that any change occurring during this time would be missed. Some increase in suction pressure was found to occur when a piece of a leaf was immersed in paraffin, before strips of tissue were torn from it, as will be shown below.

*Paraffin time factor.*

A piece of an *Iris* leaf 20 cm. long and about 1.5 cm. broad was placed in a saturated atmosphere for ten minutes to allow the natural water gradients to disappear. A small piece of the superficial tissues was then taken for a suction pressure estimation, and the remainder cut into 1 cm. lengths and immersed in paraffin. These pieces remained in paraffin on the bench, exposed to daylight. Tissue strips were taken at intervals from these larger pieces, and the suction pressure determined with the results given below.

TABLE I.

*Iris Leaf. Suction Pressure of Mesophyll Cells of Leaf during Immersion in Paraffin.*

		Time of immersion of leaf.	Suction pressure. M cane sugar.
		minutes.	
2. 10. 31.			
p.m.	2.45	0	>0.39 <0.40
	2.47	2	>0.39 <0.40
	2.50	5	0.41
	2.58	13	0.41
	3.15	30	0.41
	4.02	77	0.41
	5.45	180	0.41
	7.15	260	>0.40 <0.41
	8.25	330	0.39
3. 10. 31.			
a.m.	10.00		>0.40 <0.41
	11.30		0.41
	12.00		0.41
p.m.	1.35		0.41
	2.15		0.41
	4.00		0.41
	5.30		0.41
	6.00		0.41
	9.15		>0.39 <0.40
4. 10. 31.			
a.m.	9.00		0.39
p.m.	12.30		0.39
	2.15		Cells dying

An initial rise in suction pressure is observed which is completed in five minutes, and the value then remains constant for some hours.

This effect of the paraffin must be borne in mind when the equilibrium

suction pressure of all the cells of the entire leaf or other organ is to be measured by the cell method. The natural water gradient must be allowed to disappear by leaving the material for a few minutes in a saturated atmosphere, not by immersion in paraffin before the strips are taken. The time necessary for the cells to attain this equilibrium can be easily found by experiment; in the case of *Iris* the suction pressure of the surface mesophyll cells fell to a constant value in the first five minutes after detachment of the leaf.

*The method in detail.*

Strips of cells were isolated and immersed in paraffin and cut into small pieces a few millimetres long with a sharp scalpel. It is found that by cutting these pieces wedge-shaped and working with a cell group towards the tapering end it is quite easy to find the same group of cells again after changing the mounting fluid; without this or a similar preparation such recognition can be unexpectedly difficult.

The wedge-shaped pieces of tissue are mounted in paraffin. By using a perfectly clean slide and coverslip, and adjusting the amount of paraffin, the need for any other support for the coverslip can be eliminated. The drop of paraffin should be of such a size that it is the largest amount that can be held between the two glass surfaces; any excess will cause the preparation to slip. With practice one can obtain the mean between the insufficient amount of paraffin which flattens the material and so increases the areas of the cells, and the excess which is troublesome to deal with, though it does not affect the apparent cell size.

A group of cells in the preparation is selected and their outlines drawn under the camera lucida. The preparation is then removed from the microscope and the cover-glass lifted. The paraffin is removed from the slide with filter paper and replaced by a sugar solution. The paraffin is fairly easily displaced by the sugar solution. Any paraffin left does not seem to hinder the removal of water from the cells by the sugar solution but, unless it is entirely removed from the group of cells being studied, the paraffin in the presence of the sugar makes it difficult to see clearly the outlines of the cells. The preparation is again put under the microscope, the tissue adjusted to the same position as before, and the new area of the cells compared with the camera lucida drawing. It has been found that to make a second drawing and compare the areas as measured by a planimeter is unnecessary, and this considerably reduces the work of each estimation.

This procedure is repeated with fresh pieces of tissue until the solution is found in which there is no change in size, or the two solutions in the one of which the cells just increase and in the other just decrease.

I have used volume-normal sugar solutions made up in distilled water

and diluted in steps of 0.001 M. Slight fungal infection was found to make no appreciable difference to the osmotic pressure of the solutions, and they were therefore kept in a refrigerator at 5° C. when not in use and brought to room temperature before application to the cells, being made up freshly only every three or four weeks unless badly infected before that time. The osmotic pressures of volume-normal cane sugar solutions are given by Ursprung and Blum (2).

It is quite customary to obtain a significant expansion and contraction respectively of the cells in solutions differing only by 0.001 M (Table II). The time necessary for change in size I have found to depend very considerably on the time of year, and probably therefore on the temperature as a determining factor. During the winter, when the room temperature was in the neighbourhood of 10° C., the preparation would often give no clear response in less than an hour, but this could be hastened by warming. In the summer, with a room temperature of 20° C. or more, the response was almost immediate, as is shown in the following table.

TABLE II.

*Iris germanica.* Change in Area of Mesophyll Cells after Ten Minutes in Sugar Solution having an Osmotic Pressure very similar to the Suction Pressure. Suction Pressure 0.420 M. Room Temperature 28° C.

Cell.	Area (arbitrary units). Paraffin.	Sugar.	Increase (%).
Hypotonic sugar solution of 0.419 M.			
1	238	253	6
2	171	185	8
3	163	182	9
4	244	257	5
5	138	149	8
6	244	265	9
7	239	252	5
8	245	245	0
9	211	221	5
10	156	167	8
Hypertonic sugar solution of 0.421 M.			
1	118	110	7
2	190	172	9
3	177	170	4
4	203	194	3
5	257	236	8
6	208	201	3
7	161	157	2
8	199	188	5
9	173	163	5
10	282	282	4

A change in size of this order is easily observed in these cells.

*The gradient of suction pressure in the leaf.*

The following tests indicate that the suction pressure of the surface mesophyll of an *Iris* leaf in the intact leaf is higher than that of the internal tissue. This gradient is in the reverse direction to that found by the method of sectioning (1) and, as is to be expected, is in the direction of water movement. The suction pressure of the outermost mesophyll cells of tissue removed with the epidermis from an attached leaf by tearing, as already described, was measured. The leaf was then removed and cut into two lengths which were placed in a saturated atmosphere. The suction pressure of the surface tissues of this material was measured, after five and after fifteen minutes, with the results shown in Table III; results are based in each case on one preparation with about ten cells. The cells in one preparation were all found to give the same result.

TABLE III.

*Suction Pressure of Superficial Mesophyll Cells of:*(a) *Iris germanica*.(b) *Iris florentina*.

## Series I (a).

Mesophyll cells torn from attached leaf	0.40 M.
Strip from leaf in saturated air, after 5 minutes	0.39 M.
"      "      "      "      15      "	0.39 M.

## Series II (b).

Mesophyll cells torn from attached leaf	0.39 M.
Strip from leaf in saturated air, after 10 minutes	0.37 M.

For the first measurement in each series the cells of the first layer of mesophyll attached to strips of epidermis were torn from leaves which at the time of the removal of the tissue were still attached to the plants, so that in these leaves the natural water gradients were still in existence. The suction pressure of these mesophyll cells was determined and the value obtained was interpreted as the suction pressure resulting from the equalizing of all the suction pressures of the cells of the two layers of tissue. The second value in each series is to be regarded as the suction pressure of any cell in the leaf, when all the differences in water tension and all the natural gradients in the cells of the leaf have been caused to disappear. This value must not be called an 'average' value of the suction pressure of all the cells of the leaf since it is not dependent only on the number of cells but also on their relative sizes and the amount of the difference between the suction pressures of all the cells.

Comparison of these two values shows that the equilibrium suction pressure of the epidermis and the outermost layer of mesophyll cells is

greater than the equilibrium suction pressure reached by the equalization of the suction pressures of all the cells through the leaf. From this fact it may be deduced that the inner cells have somewhat lower suction pressures, and indeed it is to be expected that the process of water movement across the cells should be conditioned by a progressive increment in the suction pressure of the cells in the direction of water movement.

*The value of suction pressure throughout the day.*

It has been claimed that there are marked changes in suction pressure during the day. The results, however, were obtained by the method of sectioning. The tearing method was therefore used with leaves of *Iris florentina* and *Saxifraga umbrosa*. The equilibrium suction pressure of all the cells of the leaf was measured in October 1932 during a period of twenty-four hours, and the temperature of the place where the plants were growing (on the College roof) and that of the room in which the suction pressure was measured, were noted. The results are given in Table IV.

TABLE IV.

Time.	'Equilibrium' suction pressure (M cane sugar).		Temperature (° C.).	
	<i>Iris.</i>	<i>Saxifraga.</i>	Roof.	Room.
3 p.m.	0.398	—	30	26
5 "	0.398	—	29	25
7 "	0.398	0.325	26	23
9 "	0.395	0.330	24	23.5
11 "	0.393	0.330	21	23
1 a.m.	0.393	0.330	18	21
3 "	0.393	0.330	18	21
7 "	0.393	0.335	16	23
9 "	0.393	0.335	23	23
11 "	0.393	0.335	30	26
1 p.m.	0.395	0.335	32	26
3 "	0.398	0.335	32	28

The variation in suction pressure was extremely slight, being only from 10.89 atm. to 11.05 atm. in the case of *Iris*, and from 8.85 atm. to less than 9.29 atm. in the case of *Saxifraga*.

In another set of experiments (Table V) the suction pressure of the surface tissues was compared with the equilibrium suction pressure of all the cells of the leaf. The first measurements were made on the superficial mesophyll cells torn from attached leaves, and the second ones on similar cells torn from leaves after these had been detached from the plant and placed in a still atmosphere for ten minutes.

During the period of two hours the first series shows a constant difference of 0.07 atm. between the two suction pressures, which both remained constant at 10.92 atm. and 10.97 atm. respectively. In the second series

the values were slightly higher, but the difference between them was of about the same amount. A fall in suction pressure occurred towards evening and may be seen in this series to occur somewhat earlier in the day

TABLE V.

*Iris florentina.* Daily Variation in the Suction Pressure of the Cells of the Leaf.

Time.	Suction pressure (M cane sugar).		Temperature (° C.).
	After equilibrium.	Attached leaf.	
Series I. (15. 8. 32.)			
11.30 a.m.	0.394	0.396	21
12.15 p.m.	0.394	0.396	21
12.45 "	0.394	0.396	22
1.30 "	0.394	0.396	24
Series II. (12. 8. 32.)			
3 p.m.	0.396	0.398	27
5 "	0.396	0.398	27
6 "	0.396	0.397	24
7 "	0.396	0.397	24
8 "	0.396	0.396	23
9 "	0.393	0.393	21
10 "	0.393	0.393	20

in the cells from attached leaves. At 10 p.m. the two measurements gave the same result, so that the gradient of suction pressure across the leaf had apparently disappeared, or been reduced below measurable significance; this may indicate either a closing of the stomata or a fall in the evaporating power of the air. A twenty-four hour test (Table VI) showed that the low night value common to both the measurements remained constant, within measurable limits, through the night and till the following midday.

TABLE VI.

*Iris germanica.* Daily Variation in the Suction Pressure of the Cells of the Leaf.

Time.	Suction pressure (M cane sugar).		Temperature (° C.).
	After equilibrium.	Attached leaf.	
11.40 a.m.	0.414	0.420	24
12 noon	0.414	0.420	25
1 p.m.	0.414	0.420	33
3 "	0.414	0.420	35
5 "	0.414	0.416	31
7 "	0.414	0.414	28
9 "	0.414	0.414	—
11.30 p.m.	0.414	0.414	—
2.15 a.m.	0.414	0.414	—
5 "	0.414	0.414	20
7 "	0.414	0.414	20
9 "	0.414	0.414	25
11 "	0.414	0.415	26
12 noon	0.414	0.420	27



The equilibrium suction pressure of all the cells of the leaf remained unchanged over the whole time, while that of the surface tissues of the leaf rose in the later part of the morning, remained constant at this new level for some hours, and then fell again to the same value as the equilibrium suction pressure of all the cells. The value in atmospheres ranged from 11.55 atm. (0.414 M) to 11.74 atm. (0.420 M).

The plants of *Iris germanica* and *I. florentina* used in all the experiments described in this paper were growing very close together. Some forty measurements have been made on material from these plants at different times through the year and for different purposes, and the suction pressure of cells from *I. germanica* has always been greater than 0.41 and less than 0.43 M, and that of cells from *I. florentina* has ranged from 0.38 M to 0.40 M. This small range of suction pressure variation in each species is very remarkable, especially if the number of factors tending to affect the water exchange and the wide range of possible and indeed probable variation of most of these factors is considered. Plasma viscosity, permeability and sugar content of the green cells among internal factors, and soil dampness and relative humidity of the air among external ones, must vary within wide limits through the twenty-four hours and through the year. There was always some difference between the suction pressures of the cells of leaves from the two species, and this difference must be due to some internal mechanism in the plants, since conditions of soil and water and atmosphere and light must have been the same for both species where they grew close together.

#### *Suction pressure and height of leaf insertion.*

The lower epidermis of the leaves of *Ampelopsis Veitchii* can be torn away in small pieces. It carries with it groups of rather irregular mesophyll cells, which though small are convenient for suction-pressure measurements. The proportion of mesophyll cells to epidermal cells is small and variable in these strips, so that it is not admissible to draw conclusions as to the suction pressure of the two surface tissues as in the case of *Iris*, where the mesophyll cells form a continuous layer one cell deep over the epidermis. Five leaves were detached near to ground-level, and five similar leaves of about the same size and fully grown from a position 20 ft. above ground level, and the superficial tissues isolated after about ten minutes when the natural gradients had probably disappeared. The pressure determined was therefore the equilibrium suction pressure of the cells of the leaf as a whole.

Table VII shows that there is apparently some increase in suction pressure of leaves with the height of insertion, for there was a rise of 0.12 atm. when passing from ground level to a height 20 ft. (11.11 atm. to 11.23 atm.).

TABLE VII.

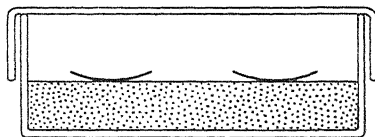
*Ampelopsis Veitchii.* The Suction Pressure and the Height of Insertion of the Leaf.

Ground-level.	Suction pressure (M cane sugar).	20-ft. level.	Suction pressure (M cane sugar).
1	0.410	1	0.414
2	0.410	2	0.414/5
3	0.410/1	3	0.414/5
4	0.410	4	0.414
5	0.410/1	5	0.414

*Behaviour of isolated tissues in saturated air.*

Pieces of a leaf were found to live for some forty-eight hours immersed in paraffin (see Table I). The cells subjected to this treatment retained a perfectly healthy appearance almost to the end, and after an initial rise in suction pressure, maintained the same pressure with remarkable constancy for two days with a nightly fall. It is difficult to state the cause of the initial rise in suction pressure, where so many complex intercellular and intracellular factors may have been affected by the isolation of a mass of tissues from the remainder of the leaf. The ultimate, almost simultaneous death of all the cells may be due to poisoning by accumulation of the  $\text{CO}_2$  of respiration. Interchange of water between the cells and the environment was of course prevented by the immersion in paraffin. Also the gas relations of the cells were probably upset by the difference in the solubilities of  $\text{CO}_2$  and oxygen in paraffin.

In the experiment just described, the gain or loss of water by the tissue, except by the intracellular processes of respiration and photosynthesis, was prevented. In the following tests, pieces of *Iris* leaf two cells thick were isolated by making a small incision with a sharp scalpel in the surface of the leaf and tearing away the easily separable material. The strips were placed on watch glasses on thick pads of water-saturated cotton wool in potato dishes, and allowed to stand on a bench near a window in daylight. The pieces of tissue were found to live for ten days or longer.



The suction pressure fell for three days, remained constant for the same period, and then rose to a considerably higher value than the original. The cells appeared perfectly healthy on the tenth day, in spite of an increased suction pressure of 1.76 atm. (an increase of 14.7 per cent. of the original value); on the eleventh day, the entire tissue was dead.

This test was repeated a little later with tissue from three leaves. In two cases the suction pressure fell during the first day and then rose. In

the third case, the measurements made on the first and second day were lost, but the shape of the curve of suction pressure for the later period suggested that the value remained unchanged for some time before rising. These results are given in Table IX.

TABLE VIII.

*Iris. Suction Pressure Changes in Isolated Tissues maintained in a Saturated Atmosphere, exposed to Daylight and to Temperature Changes in the Laboratory.*

Time (days).	1	2	3	4	5
Suction pressure (M cane sugar).	0.42-3	0.41-2	0.40-1	0.40	0.40
Suction pressure (atmospheres).	11.90	11.58	11.27	11.11	11.11
Time (days).	6	7	8	9	10
Suction pressure (M cane sugar).	0.40	0.42	0.42-3	0.44-5	0.48
Suction pressure (atmospheres).	11.11	11.74	11.90	12.56	13.66

TABLE IX.

*Iris. Suction Pressure Changes in Isolated Tissues maintained in a Saturated Atmosphere, exposed to Daylight and to Temperature Changes.*

	I.	II.	III.
Time (days).	Atmospheres.	Suction pressure. Atmospheres.	Atmospheres.
0	10.56	10.72	10.64
1	10.48	10.56	—
2	10.48	10.56	—
3	10.64	10.64	10.72
4	11.04	10.80	10.80
5	—	—	—
6	11.74	11.66	11.59
7	—	—	—
8	11.90	11.90	—
9	11.90	11.90	11.90

*The Suction Pressure Changes in Cells of Isolated Tissue maintained in a Saturated Atmosphere in the Dark, and at Constant Temperature.*

I. Strips of tissue were placed in tin-foil boats suspended from the corks of test-tubes which were nearly full of distilled water, the boats being as near as possible to the water surface. The test-tubes were kept in a water bath at 20° C. and suction pressure measurements were made each day at approximately the same time on one of these pieces of tissue.

Table X shows how the suction pressure fell slowly for two days, at the same rate. On the third day the cells were in equilibrium with distilled water, and on the fourth the remaining strips of tissue were dead.

TABLE X.

*Iris. The Suction Pressure Changes in Isolated Tissues.*

Day.	Atmospheres.
0	12.30
1	11.98
2	11.66
3	zero.

II. The suction pressure changes in strips of tissue, from the same leaf, over water, were next compared under different conditions. One strip was given the treatment just outlined, i.e., constant temperature at 20° C. and darkness. The second was allowed to remain in a potato dish on the bench, exposed to the lower temperature of the laboratory (*c.* 10° C.) and to daylight. In the dark, the suction pressure fell, but in the light, an increase in suction pressure occurred, as is seen in Table XI.

TABLE XI.

*Iris. Suction Pressure Changes in Similar Tissues, in Light and in Dark.*

Day.	Light. Atmospheres.	Dark. Atmospheres.
0	10.48	10.48
1	10.48	10.17
2	10.64	9.87
3	10.80	—

III. A number of strips of tissue, isolated with as little injury as possible from the same leaf, were submitted to different treatments. The temperature was maintained constant at 20° C. in most cases, and in two was not controlled in which it averaged about 10° C. One strip was exposed to daylight. The water content of the air was controlled in two of the five series by substituting saturated solutions of salts for the distilled water used in the previous cases.<sup>1</sup> The apparatus was the same:—tin-foil boats suspended from the corks of closed test-tubes kept in a water bath, for the constant temperature tests. The tissues to be subjected to temperature changes were kept on watch glasses on saturated cotton-wool pads in closed potato dishes on the bench. One of these was covered with black paper to exclude daylight. As Table XII shows, the cells lost water to the solutions of high osmotic pressure and their suction pressure increased, and death occurred after two days' exposure.

The entire test was repeated, using several other saturated solutions

<sup>1</sup> The solutions used to obtain vapour pressures below saturation were saturated ones of magnesium acetate and potassium bromide.

to obtain a range of the atmosphere humidities. Solutions of  $\text{NaClO}_3$  and  $\text{NaBrO}_3$  were used and similar results were obtained, as shown in Table XIII.

TABLE XII.

*Suction Pressure Changes (M Cane Sugar) in response to Different Conditions.*

Vapour pressure (mm. Hg).	Dark (20° C.).		Daylight (Temp. varying about 10° C.).		
	11.3	14.5	17.4	9.1	9.1
Day.					
0	0.380	0.380	0.380	0.380	0.380
1	0.460	0.420	0.365/70	0.370	0.380/5
2	0.490/5	0.440	0.365	0.360	0.395/40
3	—	—	0.360/5	0.350	0.410
4	—	—	0.360	0.340	0.420
5	—	—	—	0.330	0.435

TABLE XIII.

*Suction Pressure Changes in Response to Different Conditions.*

Condition.	Suction pressure.						
	Dark (20° C.). Controlled 20° C.				Daylight (varying about 10° C.).		
Vapour pressure (mm. Hg).	11.3	12.7	14.5	15.6	17	9	9
Day.							
0	0.380	0.380	0.380	0.380	0.380	0.380	0.380
1	0.465/70	0.450	0.420	0.405	0.370/5	0.370	0.380/5
2	—	0.470	0.440	0.425/30	0.365	0.360	0.395
3	—	—	—	—	0.360	0.350	0.410

The increased suction pressure of cells exposed to light may in part be due to water loss, but is probably due to some other factor. Sugar synthesis probably continues in the cells, and loss of sugar by translocation is stopped. It has not yet been possible to analyse this increase into water changes and sugar-content changes by some method of osmotic pressure determination. Such a method has still to be found; plasmolysis is useless, a freezing-point determination introduces too many errors for the results to be comparable with suction pressure measurements, and expressed sap probably bears little relation to the sap in a living cell.

#### SUMMARY.

For the method of suction pressure measurement already described (1) some points of detail are elaborated.

The effect of immersion in paraffin of portions of an *Iris* leaf is studied. There is a small initial rise during the first five minutes.

The suction pressure of the superficial mesophyll cells of leaves of *Iris* is shown to maintain a remarkably constant value in the plant, changing little during the twenty-four hours. In excerpted tissues the suction pressure shows a response to varying experimental conditions, such as light and atmospheric humidity.

An indication is obtained of a slight gradient of suction pressure across the cells of *Iris* leaves, i.e. from the water-conducting tissue to the surface tissues.

A small increase in suction pressure with the height of insertion of the leaves of *Ampelopsis Veitchii* is demonstrated.

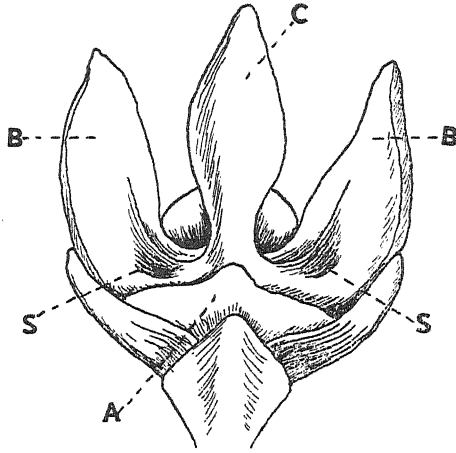
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## NOTES.

THE COLUMELLA IN THE CONE OF *DISELMA*.—*Diselma Archeri* J. D. Hook. is one of the rarer Conifers endemic in Tasmania. Its cone is small—about 2 mm.  $\times$  3 mm.—and consists of two decussate pairs of scales, the upper pair



Ovulate cone of *Diselma*, somewhat diagrammatic, showing columella.

larger and each carrying two three-winged seeds, the lower pair smaller and sterile. The general structure is shown in the accompanying drawing which is a little diagrammatic. A is one of the sterile scales, B and B the fertile scales, S and S the scars of two of the seeds and, in the centre, is the columella C to which it is desired to call attention. This structure, which is very characteristic, seems to have been overlooked in many of the systematic and general accounts of the genus. No reference is made to it by Parlatore (*Prodromus* 1868), Bentham (*Flora Australiensis* 1873), Veitch (*Manual of Conifers*), Beissner (*Nadelholzkunde* 1930), or Dallimore and Jackson (*Handbook of Conifers* 1931). Baker and Smith (*Pines of Australia* 1910), though figuring and discussing many variations in the columella of the different species of *Callitris*, do not seem to have been familiar with that of *Diselma*. These omissions are all the more remarkable, as already in 1860 J. D. Hooker (*Flora Tasmaniae*) had described and drawn this structure. In his account of the cone occur the words 'axi in centro amenti cylindraceo', while his figures 7 and 9 show the full structure of the cone with an unmistakable columella. It is peculiar, therefore, that Tarouca (*Unsere Freiland-Nadelhölzer* 1923), although reproducing most of Hooker's figures of the cone of *Diselma*, omits figures 7 and 9 which not only are the precise figures which show the columella clearly but also bring out the general cone structure more satisfactorily than any of the others.



Bentham and Hooker (Genera Plantarum 1883) include *Diselma* in *Fitzroya* and, in the general account of the combined genus, do refer to the columella in these words, 'amenti rhachis in columnam brevem oblongam (squamarum seriei quartae rudimentam) producta'. This, however, is ambiguous. In *Fitzroya* three whorls of three scales each were usually reckoned as cone scales. This explains the suggestion that the columella is the vestige of the fourth whorl. But in *Fitzroya* proper from Chili it is now well known that the columella takes the form of three diverging resiniferous columns fused at the base, to which the description above does not apply. The reference to a short column would apply to *Diselma*, but the expression 'squamarum seriei quartae rudimentam' would not suit this genus. Pilger (Gymnospermae, in Engler and Prantl 1926), among recent writers, also refers to the columella of *Diselma*—'ueber den Carp. eine kleine ovale einfache oder zweispaltige, dickliche harzige Columella'. The drawing here produced shows, as do also Hooker's figures, that the description 'kleine ovale' is hardly accurate. In the material available, which is quite plentiful, the columella is a short-stalked elongate ovate-cylindrical pointed structure; although small in actual size, when compared with the dimensions of the cone it is outstanding.

For these reasons it seemed advisable to call attention to Hooker's original reference to this important character in the *Diselma* cone.

No specimen showing a 'zweispaltige' columella was found, and this condition is possibly rare; but its occurrence is interesting. In cultivation at any rate, *Fitzroya* frequently shows variations in cone structure. Normally it possesses three large fertile scales and three smaller below, the apex of the cone axis ending in the strongly trifold columella. It may, however, show four large scales symmetrically placed, in which cases the columella is seldom trifold but strongly bifid. These cones are still distinctly Fitzroyan and quite unlike *Diselma*, but seem to emphasize the affinity between the two genera.

It is perhaps worth recording here that there exists, in the open, a group of well-grown specimens of *Diselma* in the gardens of Innacullin, Co. Cork, the property of Roland Bryce, Esq. These very interesting plants are not mentioned in the Report of the Conifer Conference of 1931.

The drawing was made by Miss M. O'Leary, M.Sc.

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**AN IMPROVED METHOD OF SOFTENING HARD WOODY TISSUES IN HYDROFLUORIC ACID UNDER PRESSURE.**—In order to cut fairly thin sections of hard tropical timbers for microscopic examination, it is necessary, in the first stage, to soften the small blocks of wood with some chemical. Many laboratories use hydrofluoric acid for this purpose. The usual procedure is to immerse the blocks in acid in gutta-percha bottles and to seal the stoppers with wax. The blocks are then kept in that state for varying lengths of time depending on the hardness of the timber. With some experience, one can make a fair guess at the time that a certain timber is likely to take to soften. Most of the hard Indian timbers take about six to

eight weeks, which is a long time, especially when one has to cut sections for urgent inquiries. During the last two years some experiments have been carried out on various timbers at the Forest Research Institute, Dehra Dun, with a view to shortening

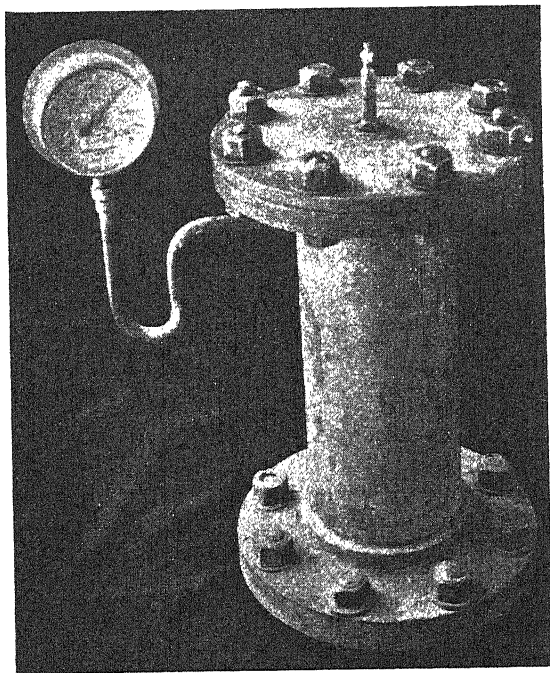


Illustration of the pressure cylinder.

this time of softening. The results obtained have been very satisfactory. Timbers which formerly used to take six weeks to soften have been softened under pressure in a week's time, and those taking eight weeks or more have been softened in ten to fifteen days.

In the present work a cylinder<sup>1</sup> similar to that employed by J. E. Lodewick (*A Shorter Celloidin Method*, Science, ii. 60, 67-8, 1924), was used with some modifications to suit the experiments. These modifications were: (a) a pressure gauge attached to the main body of the cylinder in order to indicate the pressure and to detect leakage, if any, during the process of treatment, and (b) the lining of the inside of the cylinder with lead sheeting to guard against the corroding effect of hydrofluoric acid. This gave satisfactory results.

To get uniform action of the acid, the small blocks of wood were first boiled in water for a few hours, and as far as possible all air was driven out of them. The gutta-percha bottles, containing the blocks in acid, without stoppers, were then placed in the cylinder. Having made the cylinder air-tight, pressure was applied by an ordinary foot-pump. The experiments carried out during last two years have

<sup>1</sup> My thanks are due to Mr. R. D. Tandon, Mechanical Engineer, Forest Research Institute, Dehra Dun, who made these cylinders for me.

shown that the best results are obtained by applying a pressure of about 80 lb. per sq. in. More than this tends to render the blocks brittle. It was also found that moderately soft to moderately hard timbers can be softened in two ways: either by diluting the acid<sup>1</sup> and keeping the blocks under pressure for a week, or by treating them with undiluted acid under pressure for two to four days. The former method is always better, but the latter method has been used for urgent cases with fairly satisfactory results. Hard to very hard timbers usually give no trouble when treated with undiluted acid.

At the end of the softening period the bottles are taken out from the cylinder and the blocks are washed and stored in the usual way.

During the course of experiment, over 200 blocks of some 125 species have been softened in this way. The list below shows some of the species in question and the time taken to soften them.

Name of timber species softened.	Strength of HF. %.	Number of days under pressure of 80 lb.
Large blocks (size 1" × 1" × 1").		
1. <i>Cedrella toona</i> . . . . .	40	3
2. <i>Buxus sempervirens</i> . . . . .	"	7
3. <i>Diospyros melanoxylon</i> . . . . .	"	7
4. <i>Hopea odorata</i> . . . . .	"	14
5. <i>Juglans regia</i> . . . . .	"	3
6. <i>Populus euphratica</i> . . . . .	"	3
7. <i>Santalum album</i> . . . . .	"	7
8. <i>Shorea obtusa</i> . . . . .	"	10
9. <i>Shorea robusta</i> . . . . .	"	10
10. <i>Terminalia tomentosa</i> . . . . .	"	8
Small blocks (size $\frac{1}{4}$ " × $\frac{1}{4}$ " × 1").		
1. <i>Dipterocarpus costatus</i> . . . . .	"	7
2. <i>Dipterocarpus pilosus</i> . . . . .	"	7
3. <i>Hopea odorata</i> . . . . .	"	8
4. <i>Ougeinia dalbergioides</i> . . . . .	"	9
5. <i>Terminalia tomentosa</i> . . . . .	"	5

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**A SIMPLE STAINING METHOD FOR ELEMENTARY ANATOMICAL WORK.**—The method which is the subject of the present note can be used as a temporary mounting method to give by one process a double stained preparation or by subsequent treatment of temporary mounts permanent preparations. For temporary preparations sections are mounted direct in a solution of cotton blue and magenta in lactophenol. In most instances the protoplasm, protein, and mucilage show up blue almost immediately, lignified walls and cuticle pick up the red dye fairly soon afterwards, and after a more or less protracted period the cellulose walls stain blue.

<sup>1</sup> Commercial hydrofluoric acid, 30-40 per cent.

During the period that elapses prior to attainment of the full colour the lignified walls pass through various intensities of pink or orange, and as a rule the older the lignification the more slowly staining takes place. This feature, which at first sight would seem a disadvantage, may be made of great use in teaching since it is frequently possible to demonstrate particular types of, say, xylem, by reference to colour. Thus one often sees recently mounted sections in which it is possible to point out the secondary xylem as red, the metaxylem as pink, and the protoxylem as pale pink.

In a similar manner when the element is young the walls of collenchyma and phloem tend to take up a little red in addition to blue and the result is to colour the walls a distinct mauve at first.

Preparations made for immediate examination keep well in the temporary condition since lactophenol does not evaporate, and in keeping they become more and more effectively stained. There is little tendency to overstain and differentiation is automatic, so that after a few days the coverslip may be removed, the excess of stain wiped away, and the section dehydrated, cleared in xylol, and mounted in balsam to give a permanent preparation similar to one stained with safranin and haematoxylin. Permanence of colour does not seem quite so good as this latter, however, being on a par with that of safranin and light green, but the advantage of simplicity may outweigh this objection in a method primarily for students.

As a standard concentration of stains the following is used:

1 per cent. cotton blue in lactophenol	. . .	4 c.c.
1 per cent. magenta in lactophenol	. . .	2 c.c.
Lactophenol	. . . . .	50 c.c.

Until students become used to section cutting a lower concentration of dyes in lactophenol proves more successful, since too deep a layer of stain has an appreciable light-stopping effect. Subsequently when the general run of sections is thinner the concentration may be increased with advantage to obtain more rapid staining.

A modification may also be made with advantage when the light source is very yellow. Under these circumstances extra cotton blue increases the contrast in temporary preparations, since it accelerates the staining of protein, protoplasm, &c., and also by acting as a colour filter in the mounting medium accentuates the red of lignified walls.

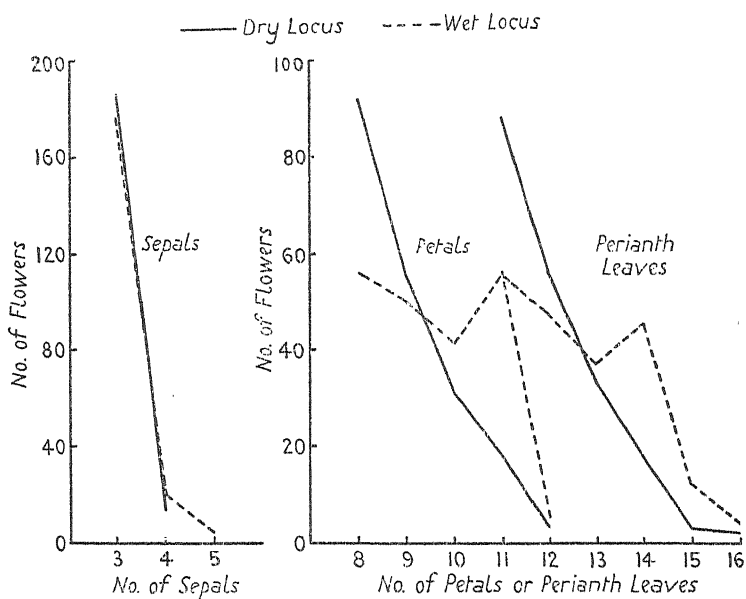
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#### VARIATION IN FLOWERS OF LESSER CELANDINE, *RANUNCULUS*

**FICARIA L.**—Two series of two hundred flowers each of the Lesser Celandine, *Ranunculus Ficaria* L., were collected on March 14th and 15th, 1931, from adjacent loci on the same (north) side of the Bedchester road, a short distance west of Fontmell Magna, Dorset. The two loci are apparently identical except in one respect—the supply of water. Both are roadside hedgebanks, one a continuation of the other; both have a generally south aspect and receive the same amount of sunshine; both are situated on a patch of Valley Gravel overlying the Gault (cf.

Sheet No. 313, Shaftesbury, of the Geological Survey). Each is backed by a hedge consisting mainly of hawthorn and hazel, the depth of the bank from hedge-bottom to road being about three feet; the lowest ten or twelve inches of this is kept trimmed



Distribution of sepals, petals, and total perianth leaves.

vertically. Their flora appear to be identical, nettles, ivy, and violets being most conspicuous amongst the grass in March, while the snail fauna of the two is the same; the presence of *Pomatias elegans* (Müller) testifies to the chalky nature of the soil.

The only visible difference between the loci is that in the case of the more easterly one there runs a small stream immediately below the foot of the bank; the more westerly locus is dry below. Celandines are practically confined in each case to the lowest eight or ten inches of the sloping bank, just above the artificially

*Correlation of Number of Petals and Number of Sepals.*

(a) Dry locus.

Number of petals.	Number of sepals.		Total.	Mean number of sepals.
	3.	4.		
8	88	4	92	3·043
9	52	4	56	3·071
10	29	2	31	3·065
11	16	2	18	3·111
12	1	2	3	3·667
Total	186	14	200	3·070
Mean number of petals	8·871	9·571	8·920	

## (b) Wet locus.

Number of petals.	No. of sepals.			Total.	Mean number of sepals.
	3.	4.	5.		
8	55	1	—	56	3·018
9	46	2	2	50	3·120
10	35	5	1	41	3·171
11	38	9	1	48	3·229
12	2	3	—	5	3·600
Total	176	20	4	200	3·140
Mean number of petals	9·352	10·550	9·750	9·480	

trimmed area, and this circumstance renders the two loci almost ideally suitable for a comparison of the flowers found in each with the object of determining the influence of an abundant and steady supply of moisture upon the number of the perianth leaves.

The whole of the perfect flowers open in each locus on the first day were collected and the number of petals and sepals of each was noted; by careful collecting the number of flowers observed for each locus was brought up on the second day to two hundred. Correlation tables were constructed showing the correlation of number of petals and number of sepals for each series, and these are given above. It is quite clear from these tables that there is in each locus a slight tendency for a high number of sepals to be associated with a high number of petals (positive correlation). Frequency polygons are shown in the Diagram, giving the distributions of petals, sepals, and total perianth leaves for the two loci. The distribution of total perianth leaves is as follows:

Number of leaves.	11.	12.	13.	14.	15.	16.	Total.	Mean.
Number of { Dry locus	88	56	33	18	3	2	200	11·990
flowers. { Wet locus	55	47	37	45	12	4	200	12·620

The distribution constants for the three variables are:

	Dry locus.			Wet locus.		
	Petals.	Sepals.	Perianth leaves.	Petals.	Sepals.	Perianth leaves.
Mean . . . . .	8·920	3·070	11·990	9·480	3·140	12·620
Standard deviation . . . . .	1·051	0·255	1·223	1·200	0·401	1·355
Coefficient of variation . . . . .	11·78	8·31	9·36	12·66	12·76	10·74
Standard error of mean . . . . .	0·0743	0·0180	0·0794	0·0848	0·0283	0·0958

Comparing the means for the two loci:

	Petals.	Sepals.	Perianth leaves.
Difference of means (Wet - Dry) . . . . .	0·560	0·070	0·630
Standard error of difference . . . . .	0·1127	0·03358	0·1244
Quotient: $\frac{\text{Difference of means}}{\text{Standard error}}$ . . . . .	4·97	2·08	5·06

Thus, the flowers of the wet locus have on the average both more petals and more sepals, and correspondingly more perianth leaves, than those of the dry locus.

In the case of the petals, the difference between the means is over one-half of a petal and is statistically significant. The same is true of the total perianth leaves, the distribution of which is influenced more by that of the petals than by that of the sepals, since the latter are much more nearly constant in number than the former. In the case of the sepals, it is noteworthy that no flowers with five sepals were found in the dry locus, whereas the wet station yielded four, or two per cent.; the mean number of sepals is higher in the wet locus than in the dry, but the difference is not great enough to be in the statistical sense significant with series of two hundred only (if the distribution of sepals remained constant with increasing number of flowers counted, the difference of 0.070 between the means would become 'significant' for series of less than nine hundred each).

The variability in the number of parts is similarly greater in the flowers of the wet locus in respect of each of the three variables; the increase of variability is greatest in the case of the sepals.

The conclusion appears to be inescapable that an increase in the supply of assimilable food to a group of plants of *R. Ficaria*, consequent upon the close proximity of water, favours the production of flowers characterized by a higher average number of petals, of total perianth leaves, and very probably of sepals, and also by a greater variability in the number of each.

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LEEK, STAFFS.

# Studies on the Transport of Nitrogenous Substances in the Cotton Plant.

## VI. Concerning Storage in the Bark.<sup>1</sup>

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With seven Figures in the Text.

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### SECTION I. INTRODUCTION.

PREVIOUS work has made it clear that downward transport through the stem of carbohydrate (9) and of nitrogen (5) from the foliage region to the root takes place in the inner region of the bark. As both materials

<sup>1</sup> Paper No. 11 from the Physiological Department of the Cotton Research Station, Trinidad.



appear to travel in the sieve-tubes (18) and as the gross phenomena seem to be identical (3), it may be inferred that the same mechanism is responsible for their movement. Examination of the concentration gradients in the *bark* of plants in a *vegetative* state has shown, however, that while carbohydrate travels to the root with a sugar gradient, nitrogen always moves against a gradient of organic crystalloid nitrogen. The gradient in protein nitrogen may be either positive or negative, but where positive, is always much smaller than the gradient of crystalloid nitrogen (4, 5).

It will be evident, if both these materials travel by a process analogous to diffusion that the negative gradient in nitrogen in the bark must be composed of a positive gradient of translocatory nitrogen upon which is superimposed a steeper negative gradient of relatively static nitrogen. Subsequent work (11) has shown that there is in fact a positive gradient in the residual nitrogen fraction and that the gradients of the other organic crystalloid fractions, notably asparagine, are markedly negative. The masking hypothesis (4) also received support from the observation that when transport was brought to a standstill, the sugar gradient in the bark disappeared, while there remained a negative gradient in crystalloid and in total nitrogen (5). In another experiment it was noted that if the normal downward direction of carbohydrate and of nitrogen transport was experimentally reversed the originally positive sugar gradient became negative and the negative nitrogen gradient was steepened. This change in the nitrogen gradient was interpreted as the reversal of an originally positive dynamic gradient superimposed on a relatively constant negative static gradient.

While such observations may indicate the existence of a negative gradient of static nitrogen in the bark, which masks a less steep positive gradient of mobile nitrogen, and are of importance in that they suggest that the negative nitrogen gradient found in the bark is not a bar to the acceptance of the Diffusion Theory of transport, they leave untouched the significance of this static gradient. To what extent does it represent structural as opposed to storage nitrogen? That it is due to storage rather than structural nitrogen is suggested by the marked increase in concentration that takes place as vegetative development proceeds (11). It will be clear, however, that the distinction between structural and storage nitrogen may be quite artificial. Moreover, the only criterion of storage is redistribution. The problem of storage of mineral elements in plants without specialized storage tissues is one that has as yet received but scant consideration. In the present paper we have attempted to unravel some of the factors responsible for the storage and distribution of nitrogen in the bark.

SECTION II. CURTAILMENT OF SUPPLY (EXPERIMENT I,  
APRIL 22ND-MAY 20TH, 1931).

In this experiment the supply of nitrogen to the roots of plants in a vegetative condition was cut off, and the effect of this curtailment on the distribution of nitrogen in the plant and on the gradients in the *bark* was then examined. It was expected, if storage as opposed to structural nitrogen is responsible for the negative gradient of static nitrogen, that the demands made by the growing tissues at the apex would lead to a withdrawal of nitrogen from the older basal region and so eliminate the negative gradient.

(a) *Procedure.* The plants were grown for a period of approximately ten weeks in a full water culture solution in a glass house. The culture solution was liberally (160 p.p.m.) supplied with nitrogen in the form of ammonium nitrate. We have found (see Experiment 3) that a further increase in nitrogen concentration in the solution used does not lead to any appreciable increase in dry weight. On the contrary there is usually luxury consumption of nitrogen and a decline in dry weight production. After ten weeks nitrogen was omitted from the solution. The nitrogen-free solution was changed weekly during the experiment.

As a result of constant pruning during the first ten weeks the 'tops' of the plants consisted only of the main stem, the leaves on the main stem, and of one internode and leaf per fruiting branch. The main stem was divided into three regions before the experiment began. These regions were demarcated with wool. The *Lower* region lay between the middle node and the root. The *Upper* region extended upwards from the middle node to that part of the stem which was judged to be too young to permit of separation into bark and wood. The region where the tissues were too immature to permit of separation into bark and wood is referred to as the *Apical* region, and is of course the only region where extension in length took place.

The actual tissues sampled were as follows :

Apical Region. Leaf lamina, petiole, and stem.

Upper Region. Leaf lamina, petiole, bark, and wood. The solitary internodes that comprised the fruiting branches were bulked with the petioles. In length they were about equal to the petioles of the main stem leaves.

Lower Region. As for Upper region.

Root Region. Bark and wood of tap-root and the fibrous roots.

The plants were graded on the basis of height before the experiment began. At each collection there were two samples, each sample containing six plants. The results are expressed on the sample basis and represent

the weights of material per 100 plants. Concentrations are expressed as grm. per 100 grm. water. The first collection was made immediately before the nitrogen supply was curtailed, the second after an interval of a week, and the third after a further period of three weeks. At the time of the third collection a collection was also made of plants to which the supply of nitrogen had not been curtailed. A number of leaves were shed from the Lower region during the experiment. These leaves were collected and were bulked with the Lower leaves on the plants at the time of the collection. The leaves shed from the plants with continued nitrogen supply were unfortunately not collected, so that the results for this group are incomplete. Carbohydrate has been taken to be equal to dry weight less 5.7 times the weight of nitrogen.

(b) *Results.* The relative changes that occurred in the weights of nitrogen and of carbohydrate in the whole plant between the first and the two subsequent collections are shown in Table I. Fully significant changes ( $P = 0.05$ ) are shown in heavy type and partially significant differences ( $P = 0.10$ ) in italics.

TABLE I.

*Relative (per cent.) Changes in Carbohydrate and in Nitrogen of Whole Plant.*

Collections.	Time in weeks.	Carbohydrate.	Nitrogen.
1-2	1	+ 26.44	- 9.30
1-3	4	+ 124.40	- 17.83

It will be noticed that while there occurred a large and fully significant increase in the weight of carbohydrate the weight of nitrogen diminished throughout the experiment. This loss of nitrogen was presumably in part due to movement into the culture solution, but loss into the atmosphere may also have played a part, for we have found that appreciable losses in nitrogen may occur from isolated leaves (cf. 1). Nitrogen starvation is suggested by the rapid drop that occurred in the weight of nitrogen per 100 grm. dry weight, the value for which dropped from 3.01 grm. at the first collection to 1.24 grm. at the third.

The changes in the weight of nitrogen in the various *Regions* between the first and subsequent collections are shown in Table II. Below the actual changes are shown the relative changes. Statistically significant changes are shown as in Table I.

The Apical region gained 6.36 grm. nitrogen during the course of the experiment. Upward movement of nitrogen into this region is thus demonstrated. It will be observed that the losses from the regions below the Apical greatly exceeded the gains by the Apical region. The relative losses were greatest from the Upper region and least from the Root. That

nitrogen was limiting the development of the Apical region is shown by the much greater gains exhibited by the group that continued to receive nitrogen. Thus while the plants without nitrogen showed during the experiment an increase of only 62.5 per cent. in the weight of nitrogen and 447 per cent. in the weight of carbohydrate in the Apical region, the group that continued to receive nitrogen registered an increase of 446 per cent. in nitrogen and 821 per cent. in carbohydrate for the same region. The weight of nitrogen per 100 gm. dry weight in this region in the former group was 1.36 gm. at the time of the third collection, while in the latter group, which was collected at the same time, it was 2.52 gm. Nitrogen starvation of the Apical region as a result of curtailment of supply is clearly indicated.

TABLE II.

*Actual (gram.) and Relative (per cent.) Changes in Nitrogen in Regions.*

Collections.	Change.	Regions.				
		Apical.	Upper.	Lower.	Root.	All below apical.
1-2	{ Actual	+ 0.20	- 4.40	- 0.60	- 0.37	- 5.37
	{ Relative	+ 1.96	- 17.13	- 7.88	- 3.08	- 11.85
1-3	{ Actual	+ 6.36	- 12.90	- 1.63	- 1.73	- 16.26
	{ Relative	+ 62.48	- 50.21	- 21.42	- 14.42	- 35.89

The changes that occurred in the distribution of nitrogen in the various tissues are shown in Fig. 1. The significant differences ( $P = 0.05$ ) are shown by the vertical lines on the right. It will be seen that the bulk of the nitrogen was present in the leaves and that the most important changes also took place in these organs. In the Apical region, leaf lamina, petioles, and stem all registered gains. In the Upper and in the Lower regions the leaf laminae lost during the experiment 58 and 74 per cent. respectively of their nitrogen, while the bark gained 75 and 80 per cent. respectively, and the wood 5 and 36 per cent. The gains by the stem tissues under conditions of starvation are remarkable. It will be observed that the petioles also registered losses. It is important to note that the nitrogen losses from the leaves of the Upper and Lower regions may not have been entirely due to starvation, for the leaves of the Upper region of the group of plants that continued to receive nitrogen showed a *net* loss of nitrogen. The loss was 26 per cent. from the plants continuously supplied with nitrogen as compared with 58 per cent. from the nitrogen starved plants. As the uptake by the former group is unknown, the actual loss cannot be gauged and may have equalled that of the latter group.

It will be evident, as there was a gain by instead of a withdrawal of nitrogen from the bark of the Upper and Lower regions, that there is no

suggestion that the normal negative gradients of nitrogen are due to storage nitrogen. The actual weights of nitrogen in the bark at each collection as well as the weights per 100 grm. water are shown in Fig. 2. It

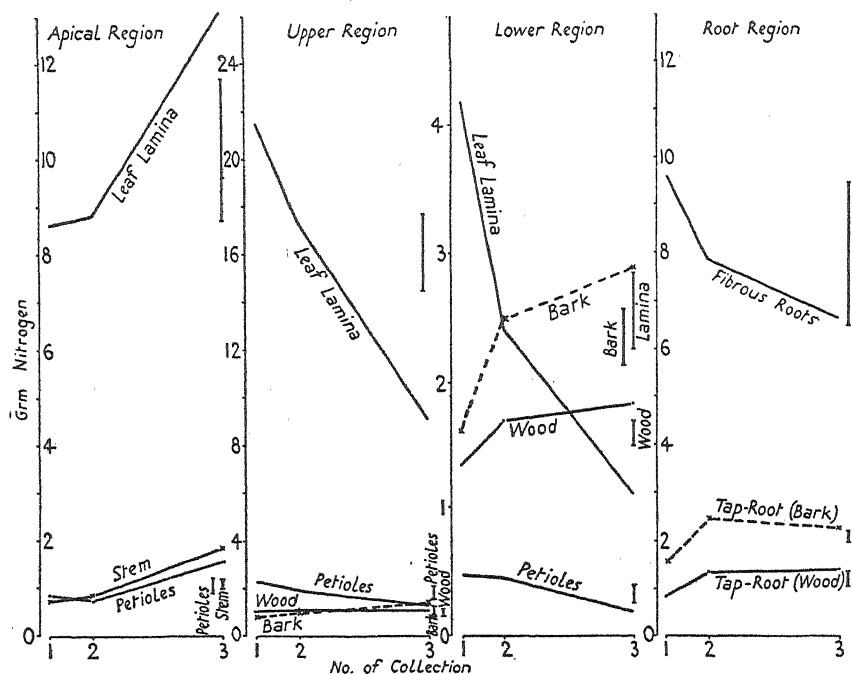


FIG. 1. Weight of nitrogen per 100 plants in various tissues.

will be seen that the concentrations increased between collections 1 and 2, and that the gradients remained negative throughout the experiment. Had the stem been defoliated, growth of and *storage* by the stem tissues might have been limited by the absence of the cambial or some other hormone produced by the leaves (20), and withdrawal of nitrogen from the bark might have occurred.

To sum up, curtailment of nitrogen to plants in a vegetative condition is accompanied by an upward movement of nitrogen from the mature leaves to the young tissues at the apex. A net loss in nitrogen also occurs from the mature leaves even when supply is not curtailed, but under these conditions the loss is *much* smaller. With supply curtailed there is a loss of nitrogen from the plant as a whole. Whether the loss occurs as a result of leakage into the culture solution or into the atmosphere or into both is not clear. Curtailment does not lead to movement of nitrogen from the stem tissues, which continue to gain nitrogen and to increase their concentration at the expense of the mature leaves, nor does the normal negative gradient exhibit any trend towards reversal, even when the development of

the young tissues at the apex is limited by nitrogen starvation. Evidence of a storage component in the bark gradient is therefore lacking.

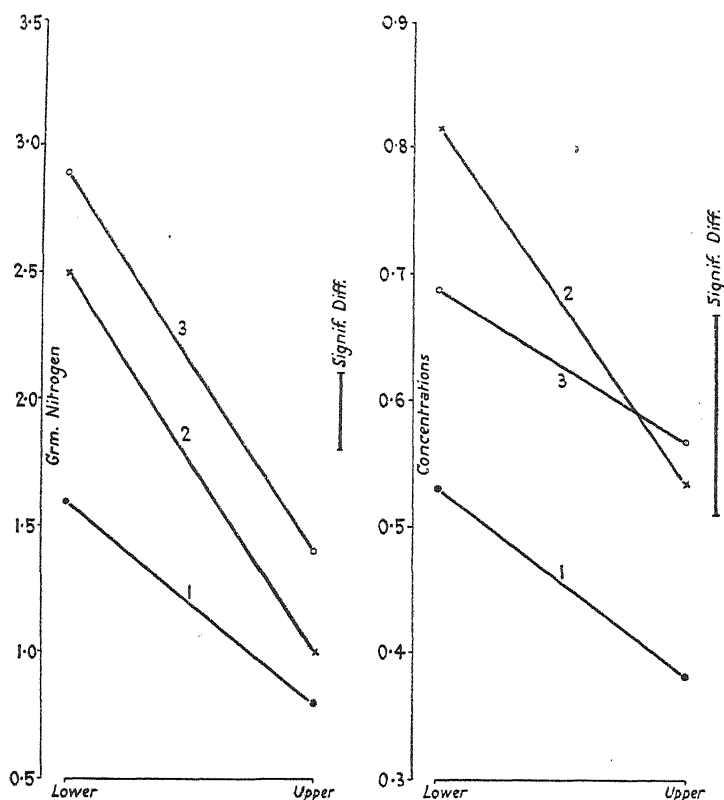


FIG. 2. Weight of nitrogen per 100 plants and concentrations in bark of Upper and Lower regions at time of three collections.

### SECTION III. BOLLING (EXPERIMENT 2, DECEMBER 1ST, 1931-MARCH 2ND, 1932).

In this experiment observations were made on the changes in the distribution of nitrogen and of calcium, and on the gradients in the bark, as the plants advanced from the vegetative through the reproductive phase. It was thought that the bolls would withdraw nitrogen from the vegetative body (6, 7, 13) and that consequently the negative gradient in the bark might be removed. As previous work (10) has pointed to calcium being relatively immobile in the phloem, it was not expected that the calcium gradients in the bark would be disturbed by the maturation of the fruits.

(a) *Procedure.* The plants were growing in the open and were rooted in a fertile silt loam. The first collection was made approximately three

months after sowing. At this time the plants, which had been flowering for about a month, consisted of the main axis and the fruiting branches, each with their leaves. On the fruiting branches there were also flower

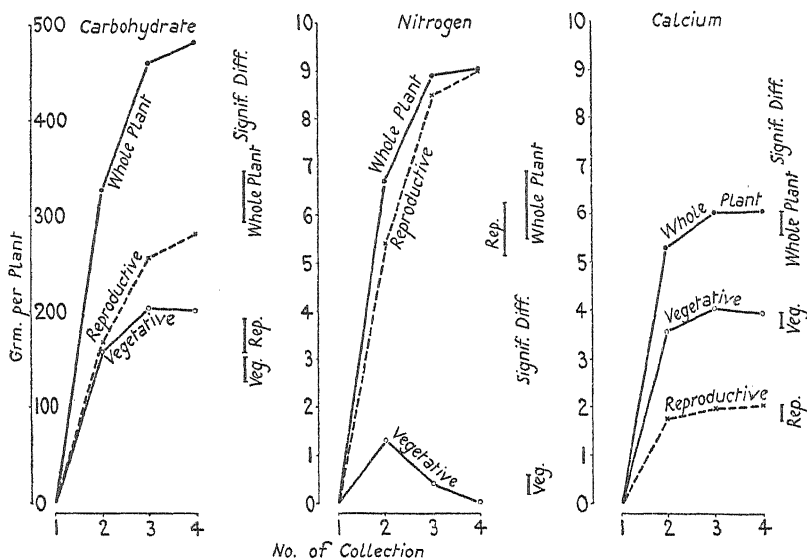


FIG. 3. Changes in weight of carbohydrate, of nitrogen, and of calcium per plant, for vegetative, reproductive, and whole plant.

buds, open flowers, and young bolls. Subsequent collections were made at intervals of a month until the termination of flowering. In all there were four collections. At each collection there were two samples. The samples contained twenty-five plants, and the results are expressed on the sample basis and represent the weights of material per single plant. Concentrations are again expressed as grm. per 100 grm. water. Grading was done on the basis of height and all the samples were drawn before the first collection.

There were, as in Experiment 1, two regions of stem where the bark concentrations were examined. The *Lower* region extended upwards from the lowest fruiting branch to the middle node of the main stem, where wool was placed. The *Upper* region lay between the middle node and that part of the plant too immature for the separation of bark and wood. The rest of the plant including roots, leaves, fruiting branches, and the reproductive parts (flower buds, flowers, bolls, and pedicels), and the stem above and below the sampled regions where the gradients in the bark were examined, was also collected. All leaves, flower buds, and bolls, which were shed during the course of the experiment, were collected and were added to the material sampled at each collection.

(b) *Results.* The changes in the weights of carbohydrate, nitrogen,

and calcium between the first and subsequent collections are recorded in Fig. 3. The results for the vegetative and for the reproductive parts and for the whole plant are shown. For the whole plant the decline in the rate of nitrogen and of calcium uptake by the root is more rapid than the decline in the rate of carbohydrate production by the leaves (cf. 17). Thus at the time of the first collection, when the plants were approximately three months old, the whole plant had absorbed 48 per cent. of its total uptake of nitrogen, 51 per cent. of its total weight of calcium, and had only produced 37 per cent. of its final weight of carbohydrate; changes in the rate of respiration are of course unknown. The cause of the difference in the rate at which the mineral elements accumulate in the plant and at which carbohydrate appears (cf. 14) to be produced is not yet clear, but is probably in some way due to the deflexion of carbohydrate from the roots to the developing fruits (cf. 6, 7, 13). It will be observed that there was no increase in the weight of carbohydrate in the vegetative part of the plant after the third collection. The difference in the relative rates of nitrogen and of calcium uptake is probably not significant; it is interesting to note, however, that Rippel (16) found that the uptake of nitrogen by the plant diminished more rapidly with increasing age than that of calcium.

The changes in the vegetative and reproductive parts of the plant are mainly of interest in that between the second and fourth collections there was an exodus of nitrogen, but not of calcium, from the vegetative to the reproductive parts of the plant. This loss in nitrogen amounted to 16 per cent. of that in the vegetative plant. It will be noticed that between the first and second collections much more nitrogen moved into the reproductive than into the vegetative parts of the plant and that the reverse obtained for calcium. It is not clear to what extent the absence of calcium transport from the vegetative into the reproductive organs is due to a low requirement of the reproductive parts and one that is therefore satisfied by the calcium in the transpiration current, and to what extent to the immobility of calcium in the phloem. It will be clear that if utilization (requirement) conditions phloem mobility, the mechanism of phloem transport and of import by the root must be different, for the uptake of non-essential elements and of essential elements in excess of requirement (luxury consumption) by the root evidently proceeds independently of requirement. Import by the root is apparently polarised, and may, like the movement of sugar from the mesophyll to vein (15), proceed against a gradient, while transport along the sieve-tubes is not and occurs in the direction of the gradient.

It is with the withdrawal of nitrogen that we are mainly concerned. The changes in the amount of nitrogen and also of calcium in the several tissues composing the vegetative plant are shown in Fig. 4. The significant differences ( $P = 0.05$ ) are shown by the vertical lines on the right. The



results are expressed as percentages of the amounts at the first collection. It will be seen that the leaf lamina, petiole, and bark all register losses of nitrogen after the second collection. The loss from the bark between the

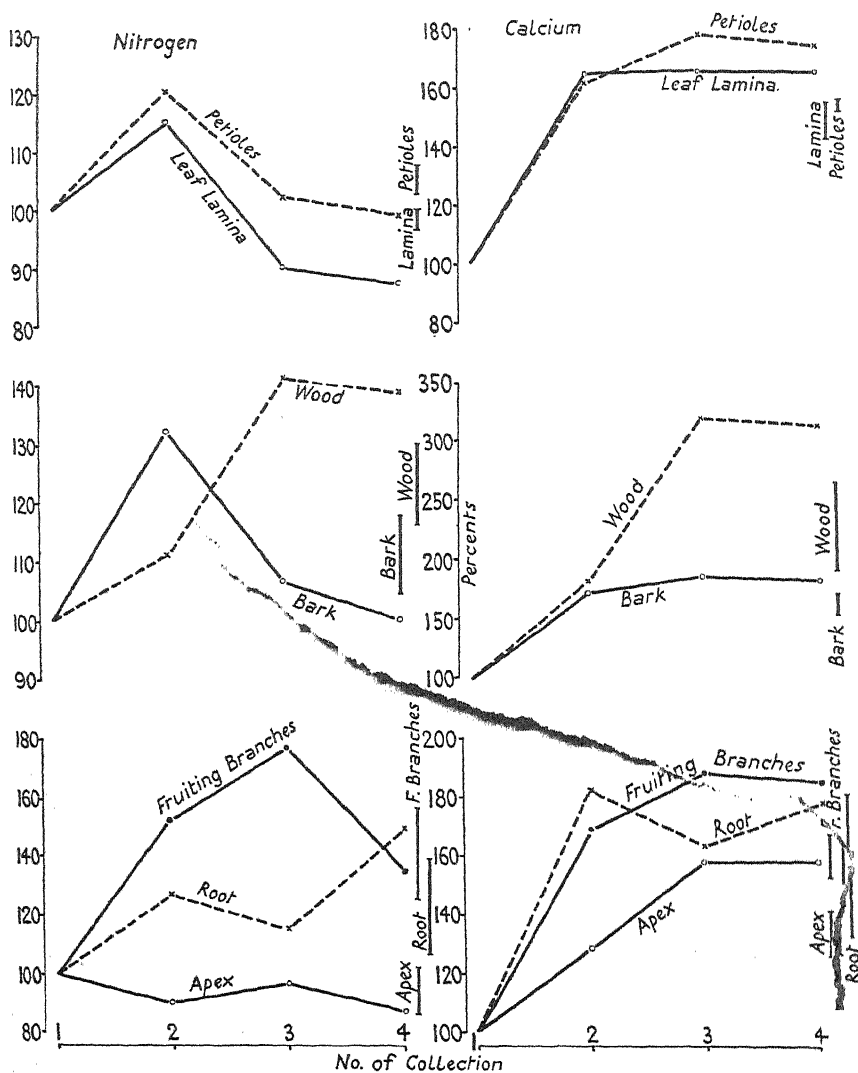


FIG. 4. Changes in weight of nitrogen and of calcium in tissues of vegetative plant expressed as percentages of Collection 1.

second and fourth collections amounted to 24 per cent. The fruiting branches show a fully significant loss of nitrogen after the third collection. The apex, that region of the main stem where the tissues were too immature for the separation of bark and wood, shows no change. The root and the

wood of the main stem register a more or less continuous gain. The results for calcium differ from those of nitrogen in that none of the tissues

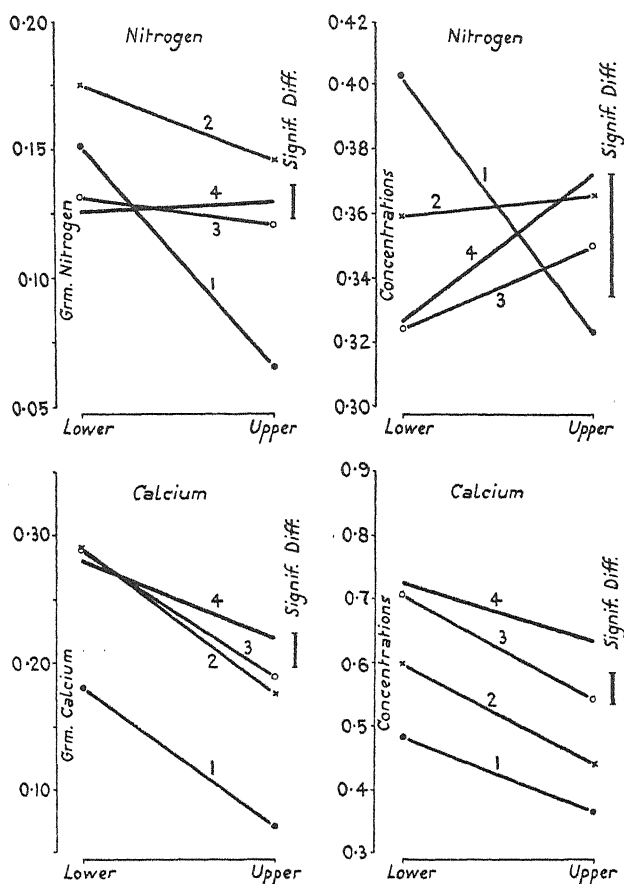


FIG. 5. Weights of nitrogen and calcium per plant (left), and concentrations (right), in Upper and Lower bark regions at time of four collections.

experienced a loss of calcium. The results emphasize the immobility of calcium (10).

The effect of these changes on the gradients in the bark is shown in Fig. 5. On the left are shown the changes in the amounts of nitrogen and calcium respectively in the Upper and Lower regions of the bark. The amount of nitrogen and of calcium in both regions increased between the first and second collections. After this there was a withdrawal of nitrogen and but little change in calcium. The Upper region does, however, show a significant increase in calcium between the second and fourth collections, but the losses from the Lower region are without significance. Between the second and fourth collections the Lower region lost 28 per cent. of its

nitrogen, while between the second and third the Upper region lost 16 per cent., these being the maximum losses. As 'withdrawal' is the only criterion of storage, it would appear that the Lower region contains a greater proportion of storage nitrogen than the less mature Upper region. It will be seen that the calcium gradients remained negative throughout, while the nitrogen gradients, though negative at the time of the first collection, gradually changed until they became positive. The facts recorded indicate that the negative gradient in nitrogen in the bark is due to the presence of a steep negative gradient of storage nitrogen that masks the positive gradient in translocatory nitrogen.

#### SECTION IV. VARIATION IN SUPPLY (EXPERIMENT 3, JANUARY 5TH, 1931).

In the previous section it was shown that the negative gradient in nitrogen in the bark of plants in a vegetative condition is reversed as a result of the withdrawal of nitrogen from the bark during boll development. From this it was inferred that the negative gradient is due to storage rather than to structural nitrogen. In the present experiment the relation between nitrogen supply to the roots and the concentration gradients in the bark is explored. It was thought that if the negative gradient in static nitrogen is due to storage or reserve nitrogen in the bark of the older regions of the stem, then variation in supply might be reflected in the steepness of the gradient. When nitrogen is supplied in excess of requirement and there is luxury consumption, the gradient might be negative; and when nitrogen is the factor controlling vegetative development the negative gradient might disappear or become positive. The gradient would in fact be a function of the nitrogen supplied to and taken up by the root.

This somewhat naïve conception of the relation between the nitrogen gradients in the bark and the supply to the roots, if tenable, would enable an estimate to be formed as to whether or no nitrogen was present in excess of requirement. The underlying assumption is that nitrogen is only stored in the bark when it is present in excess of requirement, and conversely that when it becomes a controlling factor it is all utilized and no storage takes place. There would appear, however, to be two possibilities. If storage by the vegetative plant is a function of supply, the bark gradients ought to be determined by supply. Under conditions of low supply storage would not occur and the gradients would be positive. Under these conditions, for normal boll development, continuous uptake of nitrogen by the roots would have to occur, and the rate of uptake by the root might also be a function of supply. The second possibility is that storage is unaffected by supply, so that the bark gradients might be

negative under conditions of low as well as under conditions of high supply. If storage occurs under conditions of low supply, when nitrogen is limiting the vegetative development of the plant, boll development need not be limited by nitrogen starvation after the cessation of uptake by the root. In this case, for the normal development of the bolls the rate of uptake of the root might be unaffected by supply. To sum up, if storage is a function of supply then the gradients in the bark and the rate of uptake by the root might be affected by the concentration in the solution bathing the roots. If storage takes place independently of supply, then the gradients should be negative even in cases where nitrogen is limiting vegetative development, and the rate of uptake by the root need not be affected by changes in supply.

(a) *Procedure.* The plants were grown in sand culture. There were four different concentrations of nitrogen used in the culture solutions, viz. 40, 80, 160, and 320 p.p.m. The sand was thoroughly leached at intervals of a week and fresh culture solution added. The main stem was divided into an *Upper* and a *Lower* region as in Experiment 2 and the nitrogen gradients in the bark between these regions were examined. All other parts of the plant, including roots, were collected, so that values for the weights of nitrogen and of carbohydrate are available. At the time the plants were collected they were five months old. Throughout their development they were continually pruned so that they consisted only of roots, main stem, and the leaves on the main stem. There were two samples for each treatment. Each sample contained twelve plants. The results are presented on the sample basis and represent the weights of material per ten plants. Concentrations are expressed as gm. per 100 gm. water.

(b) *Results.* In Fig. 6 we show the effect of varying the nitrogen supply to the roots on the production of carbohydrate and on nitrogen absorption. The first increment in supply caused a large (25 per cent.) increase in the weight of carbohydrate and the last an equally substantial reduction. Nitrogen uptake, on the other hand, was nearly proportional to the change in supply. It will be clear that the range of nitrogen supply extended from a state where nitrogen was limiting carbohydrate production to one where it was far in excess of requirement.

The effect of these differences in supply on the concentration gradients in the bark is shown in Fig. 7. The actual gradients are on the left of the figure and the relative on the right. The relative gradients are obtained by expressing the concentrations in the *Upper* and *Lower* regions as percentages of the mean concentrations in the two regions. It will be noticed that the gradients are negative under conditions of nitrogen deficiency as well as under conditions of excess supply. If, as we have suggested, the negative gradients are due to storage nitrogen, it would follow that nitrogen

is stored in the bark under conditions of nitrogen starvation. It will be seen that the relative gradients tend to be steeper where the supply is low than where it is high.

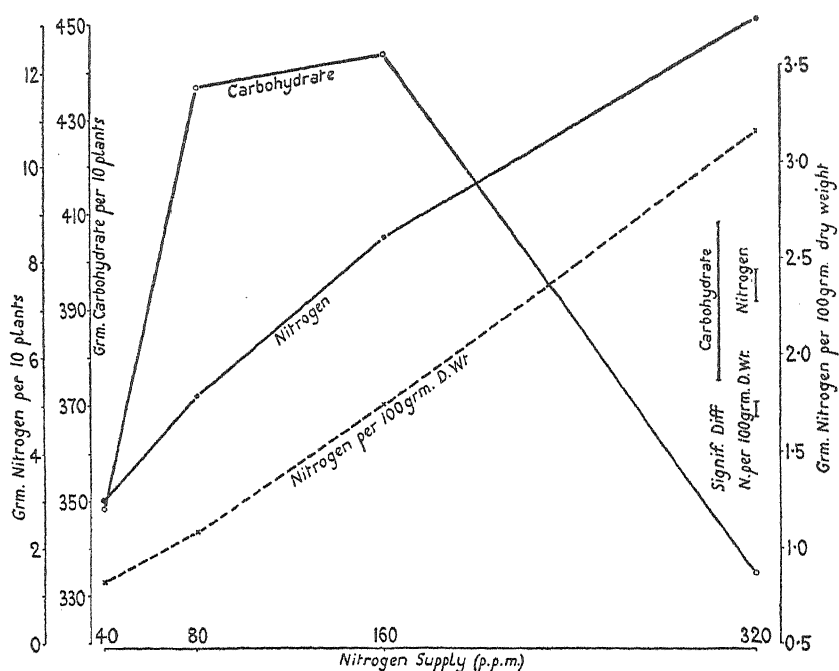


FIG. 6. Weights of carbohydrate and nitrogen per ten plants and nitrogen per 100 gm. dry weight.

TABLE III.

*Distribution of Nitrogen and of Water in the Two Regions of Bark.*

Supply p.p.m.	40.	80.	160.	320.	P = 0.05.	P = 0.10.
Per cent. in } Nitrogen	77.17	75.73	75.66	74.70	3.73	2.86
Lower region } Water	67.08	67.49	68.70	70.50	3.07	2.36

The distribution of nitrogen and of water in the two regions of the bark is shown in Table III. The values recorded represent the weights in the Lower region expressed as percentages of the total weights in the two regions. The distribution of nitrogen is affected in the same way as the relative gradients just considered. The lower the supply the greater the proportion of nitrogen in the Lower region. The distribution of water, on the other hand, was influenced in the reverse direction. These differences are small, however, and do not seem to have any general significance. In other experiments in which the supply of nitrogen to the roots was altered, some of which were carried out in water culture and some in the field, we

have found that the distribution of nitrogen is only slightly affected by variation in nitrogen supply to the roots. The distribution, moreover, is affected sometimes in one direction and sometimes in another. Nitrogen

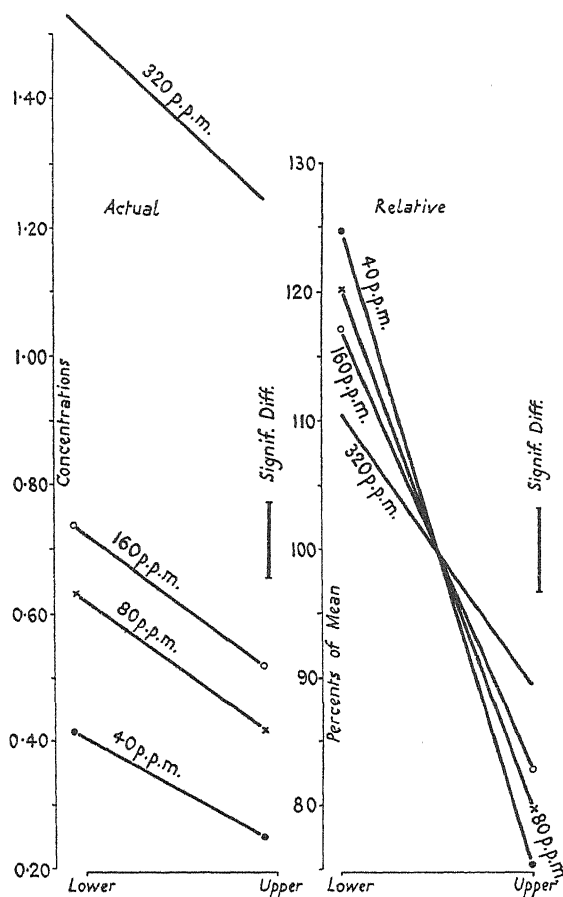


FIG. 7. Actual and relative concentrations of nitrogen in bark.

seems to be stored in the bark even when vegetative growth is being limited by a deficiency of nitrogen.

## SECTION V. DISCUSSION.

### (a) Resolution of the nitrogen gradient in the bark.

That a negative gradient in crystalloid (non-protein) and in total nitrogen is more suggestive of the *Druckstrom* of Münch (12) [cf. Dixon (2), Schumacher (18)] than of a diffusion mechanism of transport will be apparent. It should be emphasized that the negative gradient in total

nitrogen is due to crystalloid and not to protein nitrogen. If sugar on its downward journey to the root was utilized more rapidly than nitrogen, the release of water into the wood should lead to an increase in the concentration of nitrogen. In this way a negative gradient in nitrogen in the bark might arise.

In the first section of the present paper we alluded to certain observations which suggest that this negative gradient in nitrogen may be resolved into two components, a negative gradient of relatively static nitrogen which masks a positive gradient of translocatory or dynamic nitrogen. In Experiment 2 it was shown that the negative gradient disappeared during fruit development and that finally it became positive. This change in the direction of the gradient was, moreover, accompanied by a withdrawal of nitrogen from the bark, especially from the Lower region. The negative gradient is thus due to a greater proportion of storage nitrogen in the older, Lower, than in the younger, Upper, region of the bark. The concentration in the bark of the vegetative plant at different levels appears to be largely determined by the age of the tissue (11).

*(b) The immobility of calcium.*

Our conclusion as to the immobility of calcium in the phloem, based mainly on ringing experiments, is confirmed by the results presented in the present paper. Thus while the development of fruits led to a withdrawal of 16 per cent. of the nitrogen in the vegetative parts of the plant, there was coincidentally a gain instead of a loss of calcium. Moreover, the nitrogen gradient in the bark swung from markedly negative to slightly positive while the calcium gradient remained negative, and the concentration, at least in the Upper region, increased throughout development. The uptake of calcium by the root suffices for fruit development; it travels via the wood (cf. 10) and is not withdrawn from the vegetative organs by the phloem.

It is of interest to note that histochemical tests indicate that calcium is present in small amounts in the sieve-tube of the cotton plant, an observation that does not suggest a mass movement of the contents of the sieve-tube. It is, however, possible that calcium is present in the cytoplasm and not in the vacuole and that the latter only is in movement [cf. Münch (12), Schumacher (19), and Weevers (21)].

*(c) The withdrawal of nitrogen by the seed.*

The withdrawal of nitrogen from the vegetative body by the developing seed may be due, as we have suggested earlier (6), to the maintenance by rapid utilization of a low concentration of residual nitrogen in the growing ovule. There seems to be a steep gradient in this form of nitrogen between the phloem and the young seed. Similarly there appears to be

a strong positive gradient in sucrose in passing from the phloem to the ovule. As residual nitrogen appears to be the mobile form of nitrogen and sucrose of carbohydrate, the entry of these materials into the ovule would seem to be adequately explained by the Diffusion Theory.

How this withdrawal of nitrogen could occur is more difficult to explain on the *Druckstromhypothese*. Two conditions would have to be satisfied. There would in the first place have to be a positive turgor pressure gradient from sieve-tube into ovule. As the *total* sugar concentration as well as the proportion of hexoses appear to be much greater in the ovule (6) than in the phloem (9), it seems unlikely that this is so. Secondly, as food materials are utilized in the ovule, water would have to travel backwards out of the funicle via the wood, for transpiration from the ovule would be inadequate for its removal (cf. 12). Food materials not utilized would also have to be eliminated via the wood. Moreover, if calcium is immobile in the phloem, it would have to be supplied via the wood (cf. 10). There would therefore be an upward and not a backward movement of water in the xylem of the funicle. For water to move backwards in the xylem of the funicle, the suction pressure in the ovule should be low. As, however, the concentration of total sugars in the ovule is greater than elsewhere in the plant, suction pressure and turgor pressure should not both be low.

(d) *Storage and supply.*

The storage of nitrogen in the bark, as indicated by the existence of a negative gradient under conditions of nitrogen starvation, would seem to fall into line with the facts of carbohydrate storage in tubers, &c., for there can be little doubt that starch is deposited in such organs even when carbon assimilation is the controlling factor. Carbohydrate storage in the foliage leaf would appear to be different. We have found, for instance, when nitrogen supply is controlling vegetative development, that there may be an abundance of starch in the leaf *at dawn*. In this case the nitrogen in the plant body is apparently inadequate to utilize all the carbohydrate produced in the leaf during the previous day. Nitrogen and not carbohydrate is evidently limiting growth. On the other hand, we found that when nitrogen was supplied in excess of requirements (luxury consumption) the leaf was completely devoid of starch *at dawn*. The starch content of the leaf *at dawn* varied inversely, under the conditions of our experiment, with the supply of nitrogen. It would appear that in considering the relation between storage and supply that the organ of the plant must be taken into consideration. In the leaf, the normal source of supply for phloem mobile substances, storage materials may only be present at dawn, when they are present in excess of requirements. Much will, however, depend on the extent to which movement out of the leaf is polarized



(cf. 15). In other organs, which we may term 'sink' organs, storage may occur even when the particular material is the minimum or controlling factor. If we were able to characterize chemically the storage or reserve nitrogen compounds of the leaf in the way that we can starch, we might be in a position to say whether or no nitrogen was present in excess of requirement.

## SECTION VI. SUMMARY.

1. Curtailment of nitrogen supply to the roots limits the development of the young tissues and organs at the apex. Nitrogen travels from the mature leaves and petioles upwards to the young leaves and the stem. It also travels to the bark and wood of the older parts of the stem, which increase their concentration of nitrogen. As there is no withdrawal of nitrogen from the bark, even though nitrogen starvation is limiting the development of the tissues and organs at the apex, there is no suggestion that the negative gradient in the bark contains a storage component.

2. As flowering proceeds and bolls develop, nitrogen is withdrawn from the vegetative parts of the plant. For the bark this loss is relatively greater from the Lower than it is from the Upper region of the stem. Coincident with this withdrawal there is a change in the direction of the concentration gradients. At the beginning of the flowering cycle the concentration in the Lower region greatly exceeds that in the Upper and the gradient is negative. At the end of the flowering cycle the concentration in the Upper region is slightly in excess of that in the Lower region and the gradient becomes positive. From these observations it is inferred that there is a greater proportion of storage nitrogen in the Lower regions of the bark than in the Upper regions and that the original negative gradient consists of a steep negative gradient of relatively static storage nitrogen and a less marked positive gradient of translocatory nitrogen.

3. During bolling there is no withdrawal of calcium from the vegetative plant, nor from any of its tissues or organs, and the concentration gradients in the bark remain negative. It is concluded that calcium is not normally in movement in the phloem.

4. The nitrogen gradients in the bark remain markedly negative under conditions where nitrogen is limiting vegetative development. They are also markedly negative under conditions where nitrogen supply is in excess of requirement. It is inferred that the storage of nitrogen in the bark is unaffected by supply.

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# Plegetropism and the Statolith Theory.<sup>1</sup>

BY

F. M. HAINES, PH.D.

With four Figures in the Text.

RECENT work on the mechanism of geoperception has been directed in the main along such channels as are suggested on the basis of the hypothesis that curvature is the result of the production and action of growth accelerating or inhibiting hormones. Many substantial points in these directions have no doubt been established. Since, however, the production of hormones and the alterations in permeability occurring according to this conception must evidently be very secondary effects, it appeared to the writer that the more immediate effects of the stimulus merited more attention than they seemed to be receiving.

The only strictly primary effect of gravity which has been put forward appears to be that embodied in the original Statolith Theory of Haberlandt and Nemec, and the present work was undertaken with a view to obtaining a new line of evidence for or against the truth of the statolith theory. Some new lines of support for this theory have recently been developed by Hawker (1) and these together with the considerations and experiments here brought forward appear to show almost conclusively that the first effect must be of the type which would be executed by a statocyte apparatus. What remains to be shown is the nature of the connexion which must be supposed to exist between the movements of statoliths and the alterations in permeability or the production of hormones.

The present line of investigation depends upon determining the responses of organs to changes in their velocity. A movement described as *plegetropism* is found to exist in response to this type of stimulus. The considerations which led to the investigation of this movement as a means of finding support for the statolith theory will be apparent from the following line of argument.

According to the statolith theory it is supposed that the first effect of gravitational stimulation is to bring about a redistribution of the denser and less dense contents of the protoplast, denser matter or particles tending to be displaced downwards towards whichever side of the cell happens to be the lowermost. According to the same principle particles less dense

<sup>1</sup> From The Botanical Department, East London College.

than the sap in which they are suspended would be expected to be displaced upwards, the immediate subsequent effects of the redistribution in either case being possibly due to mechanical pressure on certain regions of the protoplast, to the conveyance of electrical charges, or to other conceivable causes which, however, at the present stage are purely conjectural. Leaving these for the time being out of account, however, it does appear that although the application of the statolith theory is necessarily limited to the explanation of the first effect in the chain, *this theory must be fundamentally correct in principle as far as it goes.*

We know of no effective property of gravity which could be operative in this connexion other than that it acts as a force capable of causing an acceleration in a body free to move under its influence. It would therefore appear that the only possible way in which it could exert its first effect upon the protoplast *must of necessity* and *can only* be by causing a redistribution of the denser and less dense movable constituents of the protoplast on some scale or other, denser particles or masses sinking and less dense particles or masses rising relative to the medium in which they are suspended. All other effects must be secondary to these.

It has been claimed that other possible modes of action or effects of gravity exist besides those due to its ability to accelerate a mass. A gravitational field, for example, is capable of deflecting the paths of various radiations, and a temperature effect has also been described. All such other effects of gravity which have been satisfactorily proved to occur at all, however, are infinitesimally small and in any case, as in the deflexions of the paths of radiations, resolve themselves into secondary effects of the primary capacity to accelerate a mass. The only proven effect of gravity is therefore that of accelerating a mass.

The redistribution of the particles or masses of different densities may be supposed to take place during stimulation in nature and in laboratory experiments on geotropism because the particles alone are free to be accelerated and move while the cell structure is rigidly supported and immovable. The upward thrust of the support neutralizes the force of gravity on the walls, so the movable particles are accelerated relative to the walls and the rest of the cell, and therefore relative to the medium in which they are suspended.

Now if the idea that curvatures are the indirect result of such a displacement of masses which sink or rise through the protoplasm be correct, it should theoretically be possible to cause curvatures in growing organs similar to geotropic curvatures, *not only by accelerating the particles relative to the cell, but also by accelerating the cell relative to the particles.* The effect should be the same; the mode of application of the relative acceleration different. If, therefore, a method can be devised whereby the whole cell is accelerated, but freely movable particles

do not experience a similar acceleration and this leads to curvatures, it may be taken as evidence that the curvature depends upon the displacement of movable particles or masses in the cell.

If, for instance, a sudden acceleration were applied to the cell or the whole organ, the particles, being only suspended in a more or less liquid phase, would not be accelerated to the same extent as the rigid structure but would tend by virtue of their own inertia to lag behind. The greater the acceleration of the whole cell or the more sudden its change in velocity, the more pronounced would be the lagging and the greater the resulting displacement of the particles through their own inertia, while the smaller the acceleration the smaller in proportion would be the lagging effect.

This effect can be easily demonstrated by a system of steel balls in a flat-bottomed vessel containing oil or glycerine. If such a system be moved suddenly from rest there is displacement of the balls which lag behind, but if accelerated very slowly there is practically no displacement owing to the viscosity of the medium. Similarly, if the system already in motion be stopped suddenly there is overshooting of the balls, but if slowly brought to rest there is no such displacement.

If now such a system be repeatedly accelerated quickly in one direction and slowly in the opposite direction alternately, the successive effects on the displacement will be cumulative, and eventually all the balls or particles will be found on the side of the vessel or cell to which the greater accelerating force is applied. This can be shown by repeatedly tapping the system of balls in glycerine on one side, the container being mounted on rubber in such a way that the rubber support provides a weaker restoring force, bringing the system back to its original position relatively slowly between the successive taps. The tapping is best done by means of an electric bell mechanism, when the balls will accumulate on the side which is tapped. A satisfactory model for demonstrating the movement can be made by mounting a small circular flat-bottomed glass container on one end of a flat brass arm placed horizontally and mounting the arm between two 1 inch lengths of thick-walled rubber tubing on a vertical threaded steel rod passing through a hole in the other end, nuts being placed above and below the lengths of tubing. Displacements tangential to the steel rod caused by tapping the sides of the container then cause a torque in the rubber tubing supports which restores the container to its mean position. A diagram is given in Fig. 1.

Now if we are justified in assuming the existence in the cell of a system in any way comparable in its mode of action with the above system, repeated alternate acceleration of an organ rapidly in one direction and slowly in the other should cause a similar displacement of the supposed particles in the protoplasm and therefore a curvature.

This has been tried experimentally on bean roots by a number of methods in several series of experiments, and from these it appears that sudden changes in velocity do produce curvatures. A sudden change in

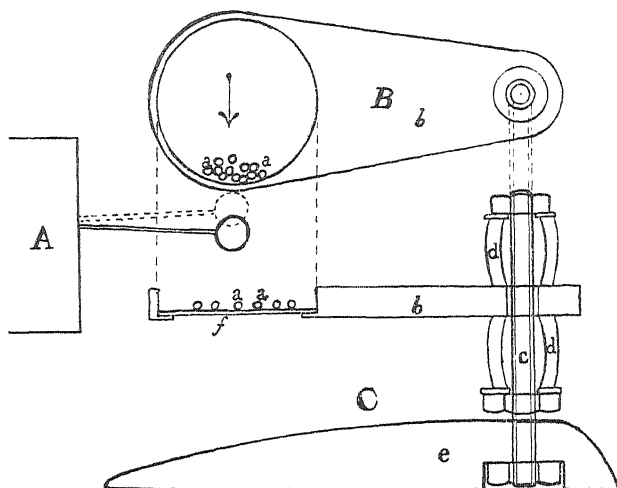


FIG. 1. Model for demonstrating movements of steel balls on tapping container. *A* and *B*, plan; *C* elevation. *A*, electric bell mechanism tapping side of circular container mounted on brass arm *b*. The container contains steel balls, *aa*, on a glass bottom, *f*. The arm, *b*, is mounted on a steel pillar, *c*, fixed vertically on a base, *e*; being loose on the pillar but held in position between the lengths of rubber tubing, *dd*. The balls, *aa*, travel to the side which is tapped.

velocity is conveniently brought about in practice by the agency of a blow on the organ, or better on its support, so curvatures brought about by this means, as it is convenient to have a separate term for them, have been termed *plegetropic* (Gr.  $\pi\lambda\eta\gamma\acute{\iota}$  = a blow). The phenomenon is therefore termed *plegetropism*. It may be defined as a growth movement due to the cumulative effect of sudden changes in velocity of the whole organ.

#### EXPERIMENTAL METHODS.

**Precautions.** In experiments to demonstrate plegetropism various special precautions are necessary.

1. When the beans are placed in a container the container must be as small and light as possible in order that its change of velocity on the impact may be sudden. The inertia must be as little as possible and it must move sharply when tapped.

2. The container must be easily free to move on the impact and mounted, for instance, on rubber which will bring it relatively slowly back to its original position between the blows.

3. The container must be perfectly level except when, as in some experiments, it is mounted on a klinostat.

4. The roots must be short and stiff at the beginning of the experiments and not be long or flexible or the tips may not undergo a sufficiently sudden change in velocity on the impact.

5. The beans for the purpose must be soaked in a vertical position as the radicles appear to be geotropic as soon as they are soaked—while they are still in the seed before emerging. The dry beans are therefore first drilled through with a small twist drill in two places and pinned through these holes to a cork slab in such a position that the radicles are exactly vertical. The whole slab is then immersed in water, being kept carefully the same way up. After twenty-four hours soaking it is transferred to moist air in the same position, the roots thus never departing from the vertical position from the time they are first wetted to the time they are placed on the experimental apparatus. It is also desirable to place moist blotting-paper on both sides of the beans while in moist air or they may later curve hydrotropically through the influence of the wet paper or cork only on one side.

Methods of experiment and results. The several series of experiments are described below, the results of all the series being given in Table I.

#### SERIES 0.

In this series steel balls in glycerine were placed in a light metal container mounted on a rubber band and on one side of which an electric bell clapper was allowed to operate. The results were invariably strongly positive, the balls always wandering to the side which was tapped.

#### SERIES 1.

Three preliminary experiments were tried with broad bean roots mounted as in Series 0. All the roots showed strongly positive curvatures, curving towards the side which was tapped.<sup>1</sup> These experiments, however, required from six to twenty-two hours' stimulation as the stimulus was relatively weak, so another method was resorted to to give a stronger stimulus.

#### SERIES 2.

In this series of experiments the bean roots were mounted in a small container (lined with wet blotting-paper) on the end of a metal arm which was pivoted at the other end and repeatedly moved in one direction by an electrically driven cam mechanism and then allowed to be pulled back

<sup>1</sup> It will be observed that in order to bring the terminology into line with that used in connexion with geotropism plegetropic curvatures are spoken of as positive when the curvature is towards the side which is tapped and negative when away from it. In both cases then the curvature is reckoned as positive when towards the side to which denser matter would be expected to be displaced on the assumption of a statolith apparatus.



TABLE I.

Series.	Material.	Number curving positively through			Number failing to curve.	Number curving negatively through			Percentage of the total number		Percentage of those curving.	
		60°-90°.	30°-60°.	0°-30°.		0°-30°.	30°-60°.	60°-90°.	pos.	neg.		
0	Steel balls	.	.	.	—	—	—	—	100	—	100	—
1	Broad beans	.	.	.	0	3	0	0	100	0	100	0
2	Broad beans	.	.	.	0	6	2	0	89	0	89	11
3	Broad beans	.	.	.	2	5	5	2	63	26	85.7	14.3
3	Controls	.	.	.	0	4	3	6	37	31.5	53.85	46.15
4	Broad beans	.	.	.	1	10	3	1	74	16	87.5	12.5
5	Broad beans in sawdust (211)	.	.	.	29	32	34	13	45	28	62.5	37.5
5	Controls	.	.	.	3	5	4	6	32.4	32.4	52	48
6	Broad beans in moist air (271)	.	.	.	16	37	38	27	52	31.4	75.8	24.2
7	Runner beans in moist air (174)	.	.	.	8	74	32	15	62	24.5	82	18
	Means of series 5-7	.	.	.	—	—	—	—	53	28	73.5%	26.5%

against a stop by a strong spring after each rotation of the cam. The essentials of the arrangement are indicated in Fig. 2.

Nine experiments were performed with this type of apparatus, the

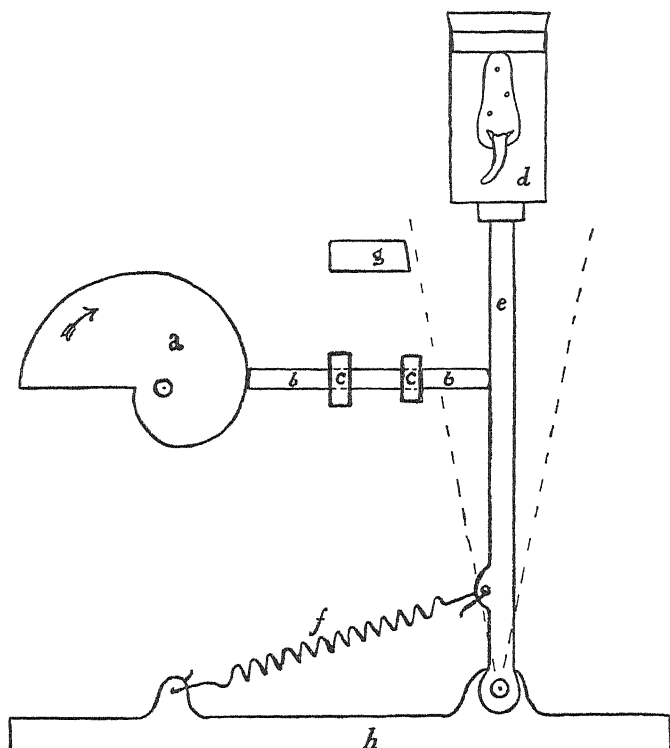


FIG. 2. Apparatus used in Series 2. The cam, *a*, is rotated approximately once a second by an electric motor with worm gearing. This causes the push-rod, *bb*, to slide in the guides, *cc*, displacing the vertical metal arm, *e*, to the right. The arm, *e*, is mounted on a base, *h*, carrying also a spring, *f*, which when *bb* is released by *a* draws the arm, *e*, to the left against the stop, *g*. The action of the stop provides the stimulus causing a bean root in the container, *d*, to curve towards the side of the stop.

mean position of the root being the vertical, with the result that one gave a slightly negative curvature, two a slightly positive curvature, and the remaining six were strongly positive.

It will be noted that in this type of experiment the sudden change in velocity causing the curvature is brought about by the stop when the system is brought to rest. The principal acting displacement of supposed particles would then be an overshooting towards the side of the stop. Curvature towards this side is therefore reckoned as positive.

### SERIES 3.

In this series a bell clapper mechanism was used as in Series 1 with the difference that the whole apparatus was mounted on the klinostat to

eliminate the simultaneous effect of gravity, but the clapper was only allowed to act directly on the roots instead of on a container. The roots were thus kept rotating while through a slip-ring contact device the bell clapper was

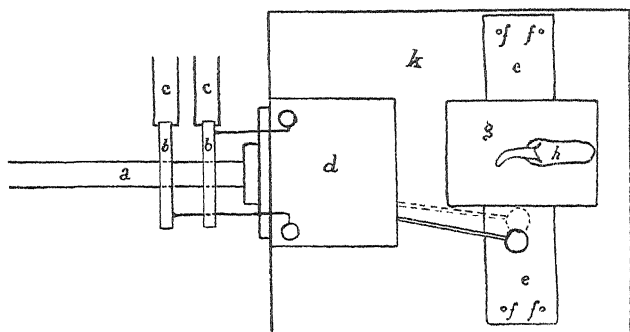


FIG. 3. Apparatus used in Series 4. The klinostat axis, *a*, carries slip rings, *bb*, making contact with the brushes, *cc*, and conveying current to the bell mechanism, *d*. The revolving axis also carries a base board, *k*, on which is mounted in addition to *d* a rubber band, *ee*, fixed to the base only at the points, *fff*. On the centre of the rubber band is mounted the container, *g*, enclosing the bean, *h*. The root curves towards the side of the container tapped by the bell mechanism, *d*.

allowed to act directly against the experimental root and simultaneously (as a control against thigmotropism) to stroke another one placed at right angles to it.

Whereas the controls showed only a relatively slight thigmotropic effect, 31.5 per cent. failing to curve and 37 per cent. curving positively as against 31.5 per cent. curving negatively, the experimental roots showed 26 per cent. failing to curve and only 11 per cent. curving negatively as against 63 per cent. curving positively, most of them strongly. Of those curving therefore only 54 per cent. were positive in the controls and 86 per cent. in the experimental roots (Table I). The experiments indicate that a plegetropic curvature is responsible but are not free from the objection that injury may be caused or that the thigmotropic stimulus may be greater in one case than in the other.

#### SERIES 4.

In this series a similar device was used to that in Series 3 with the roots mounted on the klinostat and the bell mechanism operated through a slip-ring contact, but the beans were placed in a small light container mounted on rubber and the clapper worked only on the container instead of directly on the roots (Fig. 3). This arrangement overcomes the objections to the experiments of the last series and the results are still more suggestive of the existence of the plegetropic capacity for response, 16 per cent. failing to curve, only 10 per cent. curving negatively, and 74 per cent. being positive (Table I).

In experiments of this type, however, the most marked results were obtained with a stimulation time of about sixteen to eighteen hours, the roots being examined after a further twelve hours' simple rotation on the ordinary klinostat. The experiments were therefore inconveniently long (hence the relatively small number of experiments) and in the later experiments therefore an apparatus was resorted to which would give results in a shorter time and at the same time eliminate the simultaneous effect of gravity. This apparatus is termed the *Impact Klinostat*.

#### *The Impact Klinostat.*

In this apparatus the beans are continuously rotated about a horizontal axis as on the ordinary klinostat, but instead of being mounted directly on the klinostat axis are mounted on a carriage which slides upon a guide fixed to the end of the rotating axis and placed diametrically across it. The carriage is repeatedly displaced slowly along the guide by a cam mechanism and caused to return to its original position by the action of a spring. On reaching its original position it is suddenly checked by a stop. By this means the beans, although rotating as on the ordinary klinostat to prevent curvatures due to gravity, are repeatedly accelerated alternately, relatively slowly in one direction and suddenly in the opposite direction. The relatively slow accelerations towards one side are produced by the spring and the sudden (negative) accelerations alternating with these towards the opposite side by the action of the stop. The cam causes and allows only relatively slow accelerations in both directions and really only serves to compress the spring ready for the next impact without instituting a sufficiently rapid acceleration to affect the bean or cause a curvature. The speed of the axis is ten revolutions per minute. This causes eighty impacts per minute, the cam having eight teeth. A diagram of the mechanism omitting unnecessary detail is given in Fig. 4. A shock-absorbing mechanism is incorporated in the drive in order that the impacts may not damage the worm gearing through which the axis is geared down from the electric motor which drives the klinostat. The mean tension in the spring was 2.25 kilogram. wt. and the mass of the moving parts approximately 100 grm.

With this apparatus experiments have been carried out on upwards of 700 bean roots with the mean net result that of these 28 per cent. failed to curve at all, 19 per cent. curved negatively, and 53 per cent. showed positive curvatures. That is to say, that of those curving at all 73.5 per cent. curved positively. Actually, however, this is understating the case for positive curvatures since the positive curvatures obtained were on the average much stronger and more pronounced curvatures than the negative ones. The detailed results will be found in the table (Table I).

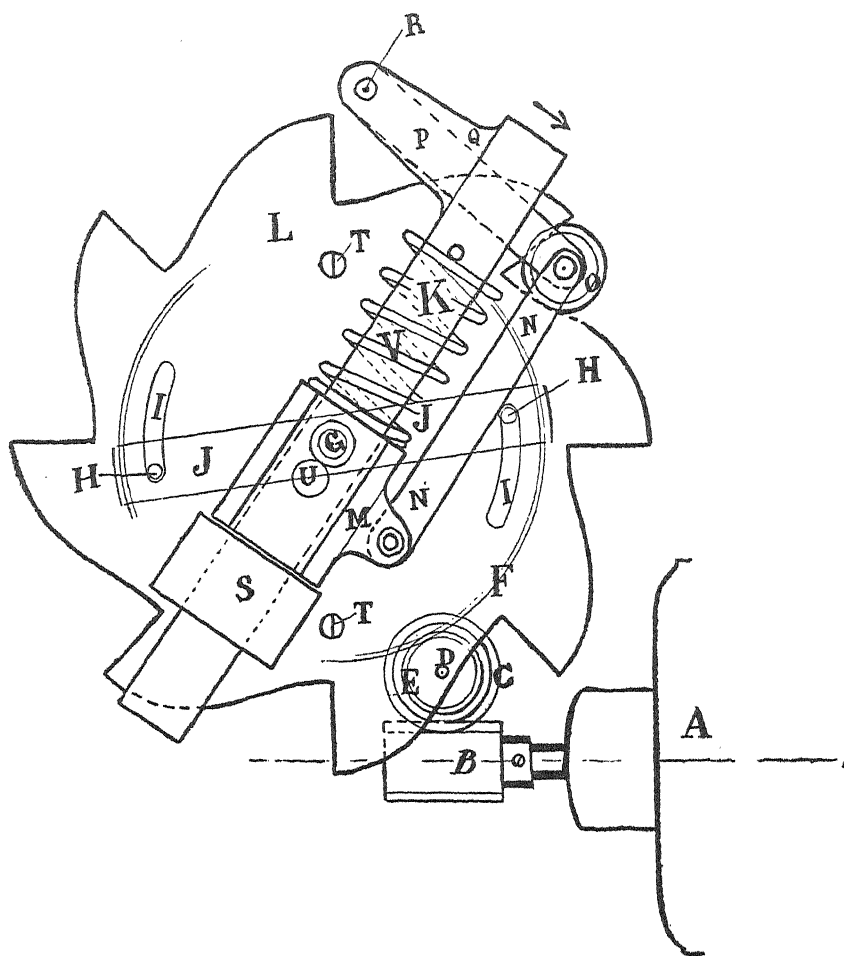


FIG. 4. Diagram to show the action of the Impact Klinostat. The spindle of the motor, *A*, carries a worm, *B*, driving the worm wheel, *C*, mounted on the countershaft, *D*, which also carries a pinion, *E*. The pinion, *E*, drives the larger toothed wheel, *F*, in which are cut the slots, *II*, and which revolves freely on the central axis, *G*. A brass arm, *JJ*, is mounted rigidly on the central axis, *G*, and carries two pins, *HH*, engaging in the slots, *II*, in the toothed wheel, *F*, so that *F* drives the central axis indirectly by driving the pins, *HH*, and therefore the arm, *J*. The central axis passes through the large cam, *L*, which has eight teeth and is fixed by the two bolts, *TT*. The axis carries on its front end the diametrically placed guide rod, *K*, on which slides the carriage, *M*, to which the container containing the beans (not shown) is fixed at *U*. As *K* revolves about the axis, *G*, the wheel, *O*, pivoted on the radius rod, *P*, rides over the teeth of the cam, repeatedly drawing *M* by the rod, *N*, along the guide away from the stop, *S*, and compressing the spring, *V*. When *O* passes over a tooth on the cam *M* is returned to the stop, *S*, by the spring, *V*, the impact providing the stimulus. The slight advancement of the axis necessary for this movement to take place sharply as *O* falls off a tooth is permitted by a movement of the pins, *HH*, along the slots, *II*. The pins immediately return to the ends of the slots as the drive catches up the slightly advanced axis. *Q*, a lug on the guide rod, *K*, to carry the pivot, *R*, of the radius rod, *P*. Stand and supports not shown.

It will be evident from the table that the results show a distinct bias in favour of positive curvature which is decidedly beyond the limits of chance and error. Since such results of plegetropic stimulation could apparently only be brought about by a statolith type of mechanism they appear to furnish evidence for the existence of a mechanism of this type in the cells and therefore to support the view that such a mechanism also plays a part in connexion with the response of roots to gravity.

A further interesting point, however, emerges from the results, and that is that although the supposed plegetropic redistribution of particles can easily be copied in various ways by mechanical and physical models as with steel balls in oil or glycerine as long as the particles are solids, it has not been possible in any way to obtain this redistributing effect of sudden changes in velocity in systems where the movable particles were gaseous or liquid. All attempts have failed to demonstrate a redistribution in this way of bubbles of gas in oils or vaseline, for example, or of drops of oil on water, and so on. This would suggest that, since roots appear to have the capacity of plegetropic response and since redistribution by this agency is not effected in systems containing droplets of liquids in other liquids of different density, the supposed redistributed particles in the case of the root must be solids and not liquids, or composed of substances relatively more solid than the surrounding medium.

It is easy to imagine that the effect in the case of liquid drops will be less pronounced since a sudden change in velocity of the medium will be expected to lead not so much to a mere jerking of the denser medium past the bubble or drop but to a deformation of the drop in which it will expand laterally in such a way as to prevent or at least impede and reduce the passage of the liquid of the medium round its edges.

The effect will therefore only be expected to be marked in the case of solid particles, and the experiments would indicate that the first effect of either a plegetropic or geotropic stimulus is to bring about the displacement of particles in the protoplasm which must be relatively solid in nature and not relatively liquid.<sup>1</sup>

It may be pointed out lastly that the ordinary experiments on centrifugal force also support the view that the first effect is a mechanical displacement of masses, since in these experiments it would again be actually the cell which is accelerated and not the particles. A more correct picture of the action of rotation is obtained if we insist not so much upon the centrifugal force which is experienced by the particles by reason of their own inertia, but upon the centripetal acceleration which is actually

<sup>1</sup> The experiments do not of course rule out the possibility of systems containing liquid particles also playing a part in the case of geotropism, but show that the observed capacities for response can be accounted for independently of these on the basis of systems containing solid particles alone, at least so far as the first stage in perception is concerned.

applied to the cell. The cell here again is really accelerated as in the present experiments, and as in these experiments the supposed particles can only be displaced by their own inertia, tending to continue to move (if more dense than the medium) tangentially away from the axis of rotation.

All the evidence therefore supports the view that curvature must be the indirect result of a mechanical redistribution of denser and less dense matter in the protoplast, the redistributed matter being relatively solid.

#### SUMMARY.

Experimental evidence is given for the existence of a plant movement known as *plegetropism* in response to sudden changes in velocity of the organ.

It appears that it must be brought about primarily through the mechanical redistribution of particles in the protoplasts.

The existence of such a movement is taken as evidence for the fundamental truth of the statolith theory of geotropism.

Since particles which are liquid in nature are not perceptibly redistributed in this way it appears likely that the particles redistributed in this movement and in geotropism are relatively solid.

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# The Effect of Light on the Respiration of Starved Leaves.

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With two Figures in the Text.

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## INTRODUCTION.

IT is well known that the respiration of the leaves detached from plants and kept in the dark decreases, at first rapidly and then slowly ; this decrease being presumably due to lack of respirable material. Ultimately the course of the curve runs almost parallel to the abscissa-axis for a considerable time, which varies according to the nature of the leaves. It has



been proved in F. F. Blackman's laboratory that the respiratory mechanism in cherry laurel deteriorates but slowly as the starvation proceeds.

While investigating further the question of starvation of the green and albino leaves of *Aralia* sp. (garden plant), it was noticed that brief exposure to light of both green and albino leaves raised the rate of respiration.

Several workers have observed the influence of light on respiration. Thus, an indirect relation between light conditions and respiration was discovered by Borodin (3). He found that the respiration of a leafy twig decreases if the twig is kept in darkness, but increases again if it is once more illuminated. He offered the explanation that by exposing the leaves to light, carbon assimilation is restarted, and thus some carbohydrates are formed, which promote the respiratory activities.

Bonnier and Mangin (2) found that there is a direct effect of light on respiration, but this effect is very slight. If plants are placed in darkness and in light alternately, there is a retarding effect on respiration, and they observed that it has no relation with photosynthetic activity, since it can be demonstrated in plants without chlorophyll.

Maximow (7) studied the effect of light on *Aspergillus niger*, and showed that it has a direct effect on respiration, which varies with the age of the culture and the nature of the nutrient medium. He observed that when the culture medium was rich in nutrient material, there was no effect of light, but when the medium was deficient the effect was markedly seen. As these plants are devoid of chlorophyll, there can be no question of assimilation. Löwshin (5), however, found no influence of diffuse light upon the rate of respiration in various fungi.

With a view to probing further into the question of influence of light on respiration, this investigation was undertaken.

After this work was begun, Masure (6) studied the effect of ultra-violet radiation on growth and respiration of pea seeds, and has shown that the rate of respiration of etiolated pea seedling is temporarily increased by subjecting them to ultra-violet radiation.

#### MATERIAL AND METHOD.

(i) *The experimental leaf.* All the leaves used throughout this investigation were taken from a particular plant, which was growing in the laboratory garden under a shady tree. Sufficient care was taken to select leaves of the same age. As far as it was practicable, the leaves were always taken from the same side of the plant. In all cases the leaves were isolated from the plant at 9.30 a.m. Soon after the leaves were picked, they were weighed and then kept in the respiration chamber with their petioles dipping in water.

(ii) *The leaf-chamber.* A rectangular museum jar was used as the leaf-chamber. Two medium size circular holes, one near the top and the other near the base, were bored on the two narrow faces of the jar:

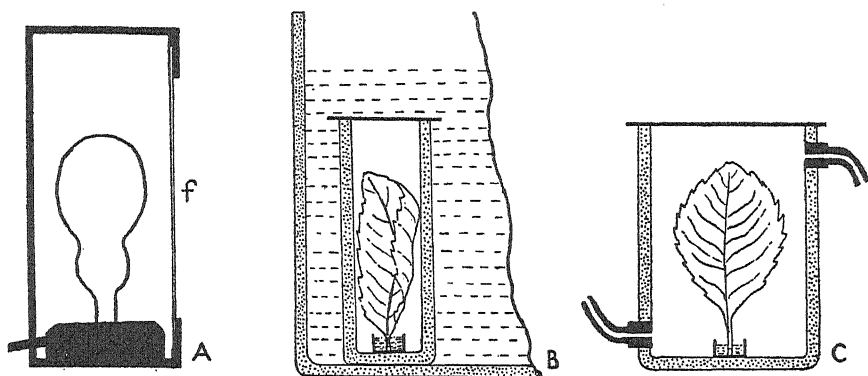


FIG. 1. Arrangement for exposure of leaves to light. A, box containing lamp; *f*, filter; B, trough full of water in which the respiration-chamber is immersed; C, front view of this respiration-chamber showing the inlet and outlet for air-current.

the former served as the outlet and the latter as the inlet for the air current when fitted with necessary glass and rubber connexions.

This glass leaf-chamber was fixed to the base of a large rectangular trough (Fig. 1) having a capacity of about 20 litres. The distance between the frontal walls of the leaf-chamber and that of the trough was 1 in. A current of water was kept flowing in the trough to keep the leaf-chamber at a constant temperature of  $28.5^{\circ}\text{C}$ .

#### EXPERIMENTAL.

(i) *Exposure of the leaves to different lights.* For exposing the leaves to white light a 60-watt Philip's Argenta bulb was used. This bulb was placed at a distance of 10 in. from the leaf in the chamber. The heat rays were cut off by a screen of running water as described above.

The blue, violet, and the red light were obtained by using Wratten filters, supplied by Messrs. Baird & Tatlock (London), Ltd. For using these filters a wooden box was used with a window facing the leaf-chamber to which the filters could be fixed when required. These filters transmit almost pure monochromatic lights. The same intensity of light was used in all cases.

The period for which the leaves were exposed to different lights varied from seven and a half minutes to two hours.

(ii) *Estimation of carbon dioxide.* The estimation of carbon dioxide was made by the usual continuous current method, using a measured quantity of baryta water for the absorption of the respired carbon dioxide,

and then volumetrically estimating the unspent baryta water with hydrochloric acid of known strength. All estimations were made under identical experimental conditions. The carbon dioxide respired was measured every two hours, and the rate was calculated per 10 gm. of fresh weight of the leaf.

(iii) *Estimation of sugar in the starving leaves before and after exposure to white light.* Two leaves practically of the same age and from the same side of the plant were picked at 9.30 a.m. They were kept, with their petioles dipping in water, in a chamber through which a continuous current of air was drawn, and which was maintained at a constant temperature of  $28.5^{\circ}\text{C}$ . After the leaves were thus treated for six hours, they were weighed and again put back in the chamber. After ninety-four hours of starvation both the leaves were taken out in dim red light. One of them, which served as the control, was at once dropped into freely boiling water, and the other was exposed to white light for 7.5 minutes, under the same experimental condition as those leaves which were kept for studying the respiration. Immediately after the exposure of the leaf to white light, it was also dropped into boiling water. The control and the experimental leaves were then treated separately. After boiling the leaves in water for over thirty minutes, they were thoroughly crushed in a mortar till a very fine paste was obtained. The water in which the leaves were boiled was used in making up the volumes of the two leaf-pastes. The filtrate which contained the sugars was freed from impurities in the usual way by adding lead acetate and subsequent de-leading. The final filtrate was concentrated on a water bath. The solutions so obtained were filtered for the last time, and each of them was made up to 25 c.c. These leaf-extracts were then tested for sugar by Calvert's method (4).

#### RESULTS.

(1) *Relation between the length of exposure and the effect.* There is no relation between the length of exposure and the amount of the after-effect. Exposure for seven and a half minutes produces as large a respiratory effect as that for two hours.

(2) *Relation to the size of the after-effect of the period of starvation preceding exposure to light.* It is found that in the early stages of starvation exposure to light does produce no rise in the respiratory rate. As the period of starvation increases, the effect appears and becomes more and more marked, and ultimately declines again. In Fig. 2, the ratios between the pre-exposure and the post-exposure rates of respiration are plotted against the period of starvation preceding exposure. It will be seen that there is a rising drift from about the 45th hour till the 100th hour, and then the ratio becomes steady till the 200th hour, to fall again with longer starvation.

(3) *Relation between the nature of light and respiration.* Exposure to red light has no effect on respiration, while blue and violet lights raise the respiratory rates in the same way as white light. In Table I the results

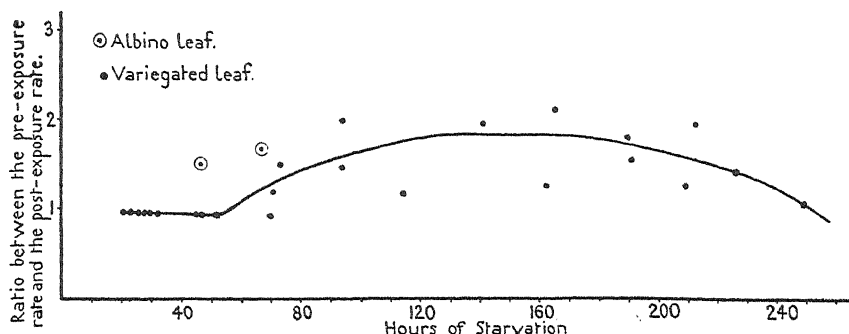


FIG. 2. Showing the drift of effect of light on respiration as starvation advances.

are arranged according to the nature of light used at about the same stage of starvation.

TABLE I.

Hours of starvation.	Nature of light used.	Time of exposure in minutes.	The after-effect. The difference between the pre- and post-exposure rates.
96	White	7.5	1.40
96	White	18.0	1.38
96	White	12.0	1.30
94	Violet	7.5	2.53
94	Violet	7.5	2.45
94	Violet	7.5	2.70
94	Blue	7.5	1.25
94	Blue	7.5	1.20
96	Red	120.0	Nil

(4) *Effect of exposure on the amount of reducing sugar in the leaf* Leaves were analysed before and after exposure to light for a definite period (7.5 minutes), and the amounts of reducing sugars noted. It was found that leaves exposed to light contained more reducing sugar than the unexposed leaves at the same stage of starvation. The results are given in Table II.

As the same result was obtained in the case of green as well as albino leaves, presumably there was no question of photosynthesis. However, to settle this question, some experiments were made on the assimilation of variegated leaves which had been starved for seventy-two and ninety-six hours respectively. The results given below (Table III) show that, at that stage of starvation, there is no assimilation even in the case of variegated

leaves. Clearly, therefore, any possibility of photosynthesis in albino leaves is precluded, although respiration is affected in both cases when the leaves are exposed to light. This assumption is strengthened when chlorophyll is extracted from the variegated and albino leaves. The albino leaves have very little chlorophyll.

TABLE II.

Weight of leaf used.	Amount of sugar in unexposed leaf after 96 hours of starvation.	Amount of sugar in exposed leaf (7·5 mins.) after 96 hrs. of starvation.	Rise of sugar due to light effect.
gr.	gram.	gram.	gram.
10	0·0006	0·0040	0·0034
10	0·0018	0·0056	0·0038
10	0·0020	0·0062	0·0042
10	0·0024	0·0055	0·0031
10	0·0021	0·0058	0·0037

TABLE III.

Hours of starvation.	Percentage of CO <sub>2</sub> used.	Vol. of gas passed through the chamber per two hours.	Total amount of CO <sub>2</sub> detected by baryta water.	Total amount of CO <sub>2</sub> respired.	Assimilation.
	%.	c.c.	c.c.	c.c.	
72	1	2000	23·4	3·4	nil
96	1	2000	23·1	3·1	nil

## DISCUSSION.

From what has been written above, the following points emerge which require explanation, namely:

(a) In the early stages of starvation up to about sixty hours, exposure to light has no effect on respiration. This period varies, but is never below forty hours. After that, exposure to blue and violet light raises the respiration, while red light has no effect.

(b) The effect goes on rising up to a certain stage of starvation and then falls.

(c) In the case of albino leaves the whole phenomenon is shifted back, i.e. the light effect is produced earlier than in the case of green or variegated leaves.

(d) Exposure to light produces an increase in the reducing sugars, although there is no likelihood of assimilation.

The fact that only blue and violet lights produce any effect indicates that the action may lie in making available some unknown reserve for the purpose of respiration. F. F. Blackman (1) has suggested a scheme for the various steps in glycolysis. It is difficult to say from the data

whether any of these steps is affected by light. Probably the hydrolytic phase is affected. This would explain the increase in the amount of reducing sugars in the cells after exposure to light. If the effect of light consists in accelerating one of the stages in glycolysis, then it is clear that when the leaf is put in the dark, the supply of respirable sugar is cut off and the rate in respiration falls.

It becomes difficult, then, to explain why light has no effect on respiration for the first forty hours or so. The rate of respiration in the *Aralia* leaves begins falling after about six hours in darkness. If the fall is simply due to depletion in the cell of respirable sugar and defective supply from the reserves, then light should produce an effect as soon as the respiration starts falling, but this is not the case. The effect of light cannot, therefore, consist merely in accelerating glycolysis.

The second possibility is the change in permeability in the cell. When the leaves are put in the dark the permeability in the cells is reduced, and respirable sugar does not find easy access to the respiratory centre. Light is known to affect permeability, and it is likely that it does so in this case. This again cannot explain why light has no effect during the early stages of starvation.

The third possibility is that light activates the enzymatic system of the cells, which is concerned with respiratory process. This explains why light effect is perceptible only after a certain period of starvation. Light does not produce any effect till the system has run down sufficiently low. It is significant that evidence has been gathered in F. F. Blackman's laboratory that in cherry-laurel leaves the respiratory system starts deteriorating after being in the dark for about forty hours, and in the experiments here recorded the effect is not perceived before that period of starvation.

One more point deserves mention here. Spoehr has found that in cacti the higher saccharides increase in the dark (8), while the hexoses decrease at the same time. This may possibly mean that (a) hexoses are used up in oxidation, or (b) part of the hexoses are built up in the dark into higher saccharides. If the latter possibility occurs in the leaves kept in the dark, then the depletion of respirable sugars in the leaf-cells, and consequent fall in the rate of respiration, is caused by

- (i) oxidation ;
- (ii) building up into higher carbohydrates ;
- (iii) sluggish hydrolysis which may be, partly at least, due to deterioration of the enzyme-system.

In conclusion, we wish to express our thanks to Professor R. H. Dastur of Bombay, for generously sending us his unpublished work on the method of sugar analysis, and to Mr. T. C. N. Singh of this college for his help in preparing the manuscript.

## SUMMARY.

The effect of light on leaves starved in the dark was studied. It is found that short exposures to light raises the rate of respiration, but this effect is not observed in the early stage of starvation extending up to forty hours or more.

Red light has no effect, while blue and violet light affect the respiratory rate in the same way as white light.

The effect increases for some time with the advance of starvation, then becomes steady, and finally declines.

Analysis shows that short exposure (7.5 minutes) to light increases the sugar content of the leaves.

This increase is not due to photosynthesis, as starved leaves have been found incapable of assimilation.

It is suggested that light increases the respiration by means of one or more of the following: hydrolysis of the reserve, activation of the enzyme system or change in permeability of the cells.

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# The Cytology of *Lobostemon* and *Echiostachys* in Relation to Taxonomy.

BY

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With twenty-five Figures in the Text.

## INTRODUCTION.

*LOBOSTEMON* is a characteristic genus of the flora of South Africa, closely related to the northern *Echium*. In fact, so close is the relationship that most of the species of *Lobostemon* have at one time or another been included in *Echium*. As recently as 1924 I. M. Johnston, in his studies on the Boraginaceae (4), failed to find any justification for retaining the genus *Lobostemon*, his conclusions being based on a careful study of the accounts of the species available at that time. Since then a detailed investigation (5), based on a first-hand knowledge of the plants, has shown that nearly all the descriptions of species are inadequate and in many cases erroneous. Consequently any accurate definition of the genus has been impossible hitherto. In addition to retaining the genus, a small group of species has been considered sufficiently distinct for promotion to generic rank, and a new genus, *Echiostachys*, has been established. The results of this investigation, which are in the press, demonstrate clearly that the separation of the South African genus is sound on both taxonomic and geographical grounds. *Lobostemon* and *Echiostachys* are not found north of 29° S. Lat., and are therefore separated from their relatives in the northern hemisphere by the vast tropical zone, a region in which, up to the present, no true *Lobostemon* has been recorded.

It is only of recent years that cytology has been employed in attempts to elucidate plant relationships. *Lobostemon* does not afford particularly favourable material, as the nuclei are small and the chromosomes are almost uniform in shape and size. However, certain points of interest emerge, and these are recorded here. Much of the value of this study lies in the fact that it was carried out concurrently with the investigation of the taxonomy and distribution of the two genera, and that opportunities arose for collecting material from a large range of localities.



## METHODS.

Carnoy's fixative was employed in the early stages of the work, but it caused considerable shrinkage, and all later fixations were made in Flemming's solution or Karpechenko's modification of Navashin's fluid. Both of these proved satisfactory, but owing to its convenience the latter was used in the majority of cases. Much of the material had to be fixed in the field, sometimes several days before a return to the laboratory was possible, and occasionally in places where water had to be carried. It is not necessary to emphasize the obvious advantages of a fixative in which material may be left for considerable periods without harm.

A thickness of  $10\ \mu$  was found to be the most satisfactory for sections. Newton's gentian violet was the principal stain used.

Root tips have not been available for study owing to a complete failure to induce germination in the laboratory. Most of the investigation has been carried out on flower buds, and where these were not obtainable, vegetative buds.

Drawings were made at bench level with a Zeiss camera lucida, using a Leitz  $\frac{1}{12}$  objective (N.A. 1.3) and a Zeiss 8 compensating eyepiece.

## RESULTS.

Both genera are characterized by normal pairing of the chromosomes at meiosis, and apart from the one instance quoted below there is no evidence of irregularity in behaviour, even in cases where hybridization was suspected. In a single plant of *Lobostemon hispidus* lagging chromosomes were frequently seen during the anaphase of the first meiotic division, and later micro-nuclei were organized in addition to nuclei of normal size. As this plant was associated with certain mutant forms it is desirable that further study of it and its mutants should be made, and the work is being continued. Thus no further reference to the matter will be made here. All other plants of *L. hispidus* showed normal behaviour.

In both *Lobostemon* and *Echiostachys* seven is the basic number of chromosomes, and no departure from this number has been observed in any of the species investigated. The results are summarized below, the localities in which the material was obtained being given in each case. The reason for this is that at an early stage of the investigation polyploidy was observed to occur within two of the species, and it was therefore considered desirable to obtain chromosome counts from as many localities as possible.

In the paper dealing with the systematic relations of the species, five natural sections are recognized in *Lobostemon*. It has only been possible to make a complete cytological examination in Section 2, where all species have been studied. In *Echiostachys* all three species have been examined.

LOBOSTEMON.

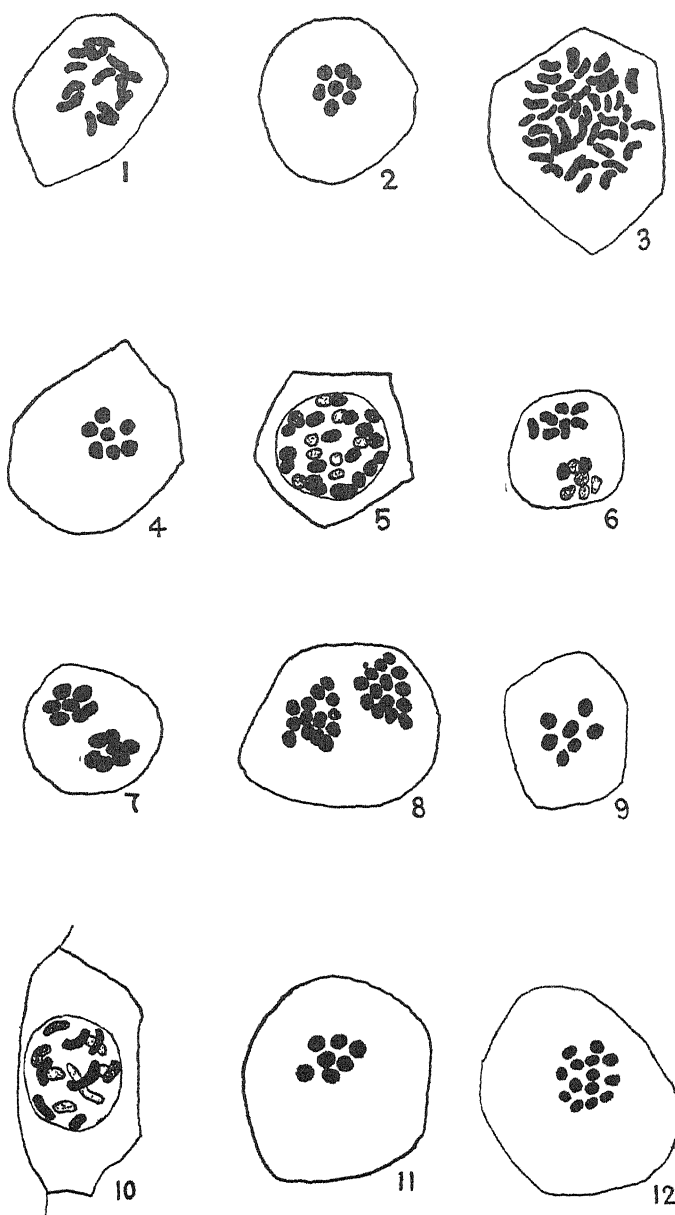
						Chromosome numbers.	
						Somatic.	Gametic.
<i>Section 1. Echioides.</i>							
<i>L. echioides</i> (Namaqualand)	.	.	.	.	.	14	7
<i>L. echioides</i> (Riversdale)	.	.	.	.	.	14	—
<i>L. echioides</i> (Ladismith)	.	.	.	.	.	42	—
<i>L. paniculatus</i> (Ladismith)	.	.	.	.	.	14	7
<i>L. Bolusii</i> (Gordon's Bay)	.	.	.	.	.	28	—
<i>Section 2. Trichotomi.</i>							
<i>L. trichotomus</i> (Hercules' Pillar, Paarl Div.)	.	.	.	.	.	14	—
<i>L. trichotomus</i> (Tulbagh)	.	.	.	.	.	—	7
<i>L. trichotomus</i> (Pakhuis Pass)	.	.	.	.	.	—	7
<i>L. paniculaeformis</i> (Darling)	.	.	.	.	.	—	7
<i>L. hispidus</i> (Durbanville)	.	.	.	.	.	—	14
<i>L. laevigatus</i> (Pakhuis Pass)	.	.	.	.	.	—	7
<i>L. laevigatus</i> (Karoo Poort)	.	.	.	.	.	14	—
<i>L. laevigatus</i> (Cederberg)	.	.	.	.	.	14	—
Giant form							
<i>L. laevigatus</i> (Cederberg)	.	.	.	.	.	—	7
Intermediate between this species and <i>L. glaucophyllus</i>							
<i>L. glaucophyllus</i> (Namaqualand)	.	.	.	.	.	—	7
<i>L. glaucophyllus</i> (Van Rhyns Pass)	.	.	.	.	.	—	14
<i>L. glaucophyllus</i> (Grey's Pass)	.	.	.	.	.	—	14
<i>L. glaucophyllus</i> (Cederberg)	.	.	.	.	.	14	—
<i>L. glaucophyllus</i> (Tulbagh)	.	.	.	.	.	—	14
<i>L. glaucophyllus</i> (Cape Peninsula)	.	.	.	.	.	—	14
<i>L. hottentoticus</i> (Sir Lowry Pass)	.	.	.	.	.	14	7
<i>L. Pearsonii</i> (Namaqualand)	.	.	.	.	.	14	—
<i>Section 3. Argentei.</i>							
<i>L. argenteus</i> (Cape Peninsula)	.	.	.	.	.	—	7
<i>Section 4. Fructicosi.</i>							
<i>L. fructicosus</i> (Cape Peninsula)	.	.	.	.	.	14	7
<i>L. curvifolius</i> (Houw Hoek)	.	.	.	.	.	14	—
<i>L. decornis</i> (Ladismith)	.	.	.	.	.	28	—
<i>Section 5. Grandiflora.</i>							
<i>L. montanus</i> (Cape Peninsula)	.	.	.	.	.	—	7

ECHIOSTACHYS.

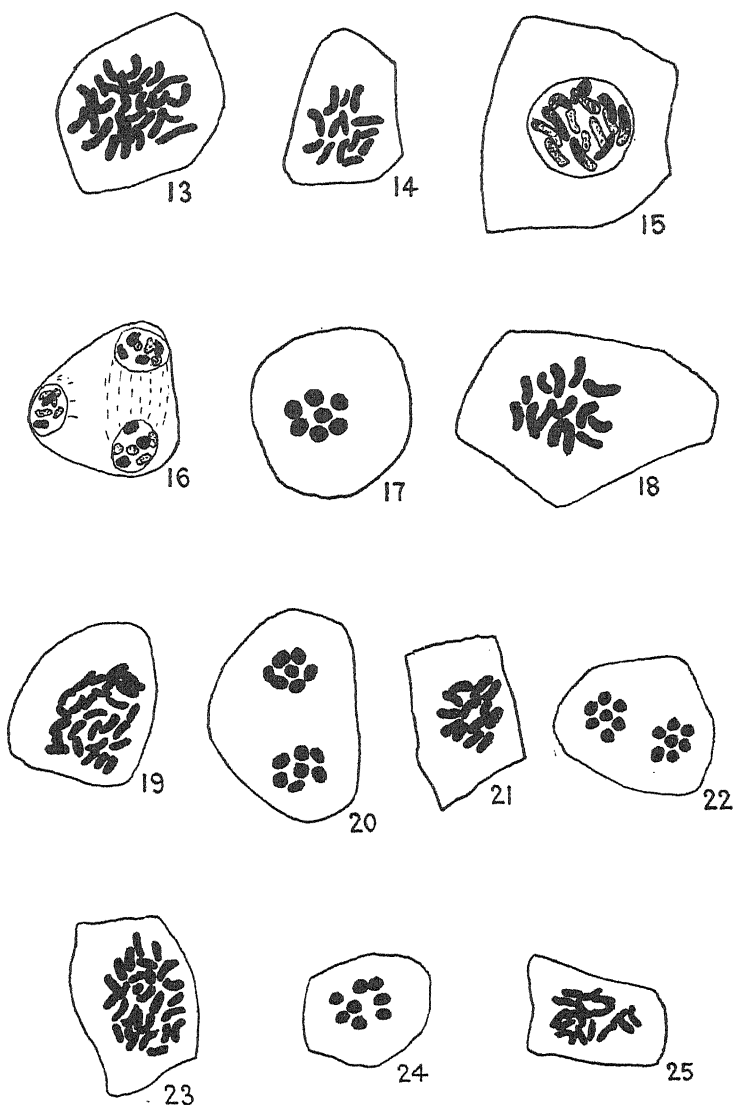
<i>E. incanus</i> (Sir Lowry Pass)	.	.	.	.	.	14	—
<i>E. ecklonianus</i> (Sir Lowry Pass)	.	.	.	.	.	—	7
<i>E. spicatus</i> (Saldanha Bay)	.	.	.	.	.	28	—

The majority of species will be seen to be diploid, but several tetraploid and one hexaploid have been recorded up to the present.

The chief point of interest lies in the occurrence of polyploidy within two of the species, viz. *L. glaucophyllus* and *L. echioides*. In both cases it is impossible to separate the polyploid from the diploid forms on any definite morphological characters, but geographically they occupy distinct regions. As the evidence is more complete in the case of *L. glaucophyllus*, this case will be chosen for illustration.



FIGS. 1-12. 1, 2. *Lobostemon echinoides* (Garies): 1. Mitosis in young anther.  $2n = 14$ .  $\times 2000$ ; 2. Metaphase of first meiotic division.  $n = 7$ .  $\times 2000$ . 3. *L. echinoides* (Ladismith). Mitosis in stem apex.  $2n = 42$ .  $\times 2000$ . 4. *L. paniculatus*. Metaphase of first meiotic division.  $n = 7$ .  $\times 2000$ . 5. *L. Bolusii*. Mitosis in young ovule.  $2n = 28$ .  $\times 2000$ . 6. *L. trichotomus*. Metaphase of second meiotic division.  $n = 7$ .  $\times 2000$ . 7. *L. paniculaeformis*. Metaphase of second meiotic division.  $n = 7$ .  $\times 2000$ . 8. *L. hispidus*. Metaphase of second meiotic division.  $n = 14$ .  $\times 2000$ . 9, 10. *L. laevigatus*: 9. Metaphase of second meiotic division.  $n = 14$ .  $\times 2000$ ; 10. Giant form. Mitosis in young anther.  $2n = 14$ .  $\times 2000$ . 11. *L. glaucophyllus* (Garies). Metaphase of first meiotic division.  $n = 7$ .  $\times 2000$ . 12. *L. glaucophyllus* (Van Rhyns Pass). Metaphase of first meiotic division.  $n = 14$ .  $\times 2000$ .

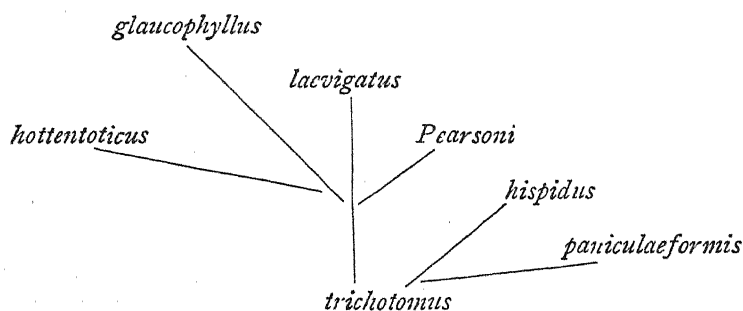


FIGS. 13-25. 13. *L. glaucophyllus* (Cape Peninsula). Mitosis in stem apex.  $2n = 28$ .  $\times 2000$ . 14. *L. hottentoticus*. Mitosis in young anther.  $2n = 14$ .  $\times 2000$ . 15. *L. Pearsonii*. Mitosis in young anther.  $2n = 14$ .  $\times 2000$ . 16. *L. argenteus*. End of second meiotic division.  $n = 7$ .  $\times 2000$ . 17. *L. fruticosus*. Metaphase of first meiotic division.  $n = 7$ .  $\times 2000$ . 18. *L. curvifolius*. Mitosis in young anther.  $2n = 14$ .  $\times 2000$ . 19. *L. decorus*. Mitosis in young anther.  $2n = 28$ .  $\times 2000$ . 20. *L. montanus*. Metaphase of second meiotic division.  $n = 7$ .  $\times 2000$ . 21. *Echiostachys incanus*. Mitosis in young ovule.  $2n = 14$ .  $\times 2000$ . 22. *Echiostachys ecklonianus*. Metaphase of second meiotic division.  $n = 7$ .  $\times 2000$ . 23. *Echiostachys spicatus*. Mitosis in young ovule.  $2n = 28$ .  $\times 2000$ . 24, 25. *Echium fastuosum*: 24. Metaphase of first meiotic division.  $n = 8$ .  $\times 2000$ ; 25. Mitosis in young ovule.  $2n = 16$ .  $\times 2000$ .

This species is confined to the western part of the Cape Province, chiefly on the lower mountain slopes. The mountains of this region run roughly north and south, and are composed of quartzitic rocks belonging to the Table Mountain series. From the Cape Peninsula to a point a little north of Van Rhyns Pass, where the Table Mountain series peters out, *L. glaucophyllus* is found wherever this geological formation occurs. Between Van Rhyns Pass and Namaqualand lies a desolate area of a desert-like character, about seventy miles in extent, where no *Lobostemons* occur. In Namaqualand, however, the Kamiesberg reach an altitude of over 5,000 feet, and at Leliefontein a rainfall averaging more than 14 inches a year has been recorded.<sup>1</sup> These more favourable conditions are reflected by the flora, which is once more of the type characteristic of the south-western coastal belt. Here on the sandy soil produced by granitic rocks *L. glaucophyllus* reappears. All these Namaqualand plants are diploid. The plants from Van Rhyns Pass southwards are tetraploid with the exception of a diploid plant collected at Algeria in the Cederberg. The problem raised by this plant is far from settled. At Algeria *L. glaucophyllus* and *L. laevigatus* (a closely allied diploid species) grade into one another in a bewildering series of forms reminiscent of the 'hybrid swarms' recorded by Allan in New Zealand (1). *L. laevigatus* occurs normally at higher altitudes than *L. glaucophyllus*, but where their areas overlap intermediates abound. Unfortunately, this region is mountainous and difficult of access, so it has not been possible to obtain further supplies of material, and until such supplies are available it cannot be said whether the plant recorded was exceptional or typical of *L. glaucophyllus* in that neighbourhood.

A comparison of Figs. 11 and 12 shows that the chromosomes at metaphase of the first meiotic division are larger in the diploid than in the tetraploid.

A comparative study of the floral and vegetative features in Section 2 of *Lobostemon* resulted in the following scheme being drawn up to indicate the interrelations of the species:



<sup>1</sup> I am indebted to the Rev. J. A. George for this figure. It is based on readings taken at Leliefontein during the last twenty-four years.

Along both lines progressive zygomorphism of the corolla and stamens is the dominant note, but the '*hispidus*' line retains the hairiness of *L. trichotomus*, while the '*glaucophyllus*' line shows a strong tendency to become glabrous. As in the case of all evolutionary problems, it is possible to read the series as one of reduction from a zygomorphic to a regular type of flower, instead of as a progressive one. The cytology of the section, however, gives emphatic support to the scheme as outlined above, as the two most zygomorphic species are both tetraploid (or largely so in the case of *L. glaucophyllus*), all the rest being diploid.

In a discussion on polyploids Darlington (2) states that a polyploid form, derived directly from a diploid by simple doubling, is normally larger than the form from which it is derived. In other words, gigantism is the normal consequence of polyploidy unless some modifying factor is introduced. Throughout *Lobostemon* polyploidy is not accompanied by increase in size, in fact in the majority of cases the polyploid species are small. That some factor controlling size operates in *Lobostemon* is suggested by a study of these plants in the field. In a paper on the taxonomy of *Lobostemon*, to which reference has been made previously, the author has shown that many species of *Lobostemon* are prone to produce mutant forms differing from the type merely in some size relation. Thus the most common type of mutant is a small microphyllous form in which both floral and vegetative axes are condensed. Gigantism occurs fairly frequently. In neither of these is there any change in chromosome number, thus showing clearly that in this genus there are factors controlling size which have no relation to the number of chromosomes.

In contrast with *Lobostemon* it is of considerable interest to find that in the small genus *Echiostachys* polyploidy and gigantism are associated. *E. spicatus*, the tetraploid, is larger in all its parts than the two diploid species.

As far as the writer is aware little work has been done on chromosome numbers in the Boraginaceae. Tischler (7) refers to four known examples, in none of which is the basic number seven. Gaiser (3) gives no further information. In a recent paper by S. G. Smith (6) an account is given of the cytology of the genus *Anchusa* in relation to taxonomy. Here he finds that in the genus as defined recently by Johnston (4), eight is the basic number. Two species which on taxonomic grounds Johnston has removed to other genera do not have this basic number, which fact is taken as supporting Johnston's contention that these species have not been correctly placed in *Anchusa*.

It is unfortunate that no published information is available with regard to chromosome numbers in *Echium*. No *Echium* is native in South Africa, but *Echium fastuosum* Salisb. (the Pride of Teneriffe) is a plant commonly grown in gardens in Cape Town. Here the gametic number of chromo-

somes is eight (Figs. 24, 25). If eight be the basic number for the genus, then, applying the argument used by S. G. Smith in the case of *Anchusa*, *Lobostemon* must be considered generically distinct from *Echium*. This is the writer's view based on other grounds, and cytology appears to offer confirmation.

#### SUMMARY.

1. *Lobostemon* and *Echiostachys* are purely South African genera closely related to *Echium*.
2. Both these genera have seven as the basic number of chromosomes, and both show polyploidy.
3. Polyploidy occurs within two of the species. No morphological distinctions can be made between the diploid and polyploid forms, but they occupy different geographical areas.
4. In the one section of *Lobostemon* in which it has been possible to make a cytological examination of all the species, support is forthcoming for the grouping of species arrived at from other considerations.
5. In *Lobostemon* factors governing size relations are independent of polyploidy, but in *Echiostachys* gigantism is associated with polyploidy.
6. Eight is probably the basic number of chromosomes in *Echium*. This supports the view that *Lobostemon* and *Echiostachys* are generically distinct from *Echium*.

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# Investigation of Factors Affecting Advance of Certain 'Apple-spot' Fungi within the Host Tissue.

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With nineteen Figures in the Text.

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## I. INTRODUCTION.

FUNGAL spotting of apples is prevalent on sweet varieties, but may occur on sour varieties late in the storage season. Its distribution is therefore associated with the lower levels of acid found in sweet, as opposed to sour, varieties after a long period of storage. There are, however, other chemical factors to be considered such as sugar content, which varies with variety and age of fruit, and nitrogen content, which may show wide variation in a single variety. Variations in nitrogen content are reflected in the rate of advance of certain fungal species in the host tissues, as demonstrated by Horne (5-10).

Recently Seth (13) applied cultural methods to the problem of the relationship between fungal advance and chemical composition of fruit, and found that advance may be conditioned by acid and sugar content. Differences in concentration of acid and sugar will also account for the variations in relative attacking power of fungal strains associated with variety and age of fruit described by Horne (9) and Das Gupta (2, 3).





inoculated from the stock tubes and kept at 20° C. for one week. Experimental series were then inoculated with millimetre cubes of the standard medium plate culture, taken from the zone about 1 cm. inwards from the margin of growth. It was thought preferable to use spores for the inoculation of plates with *Fusarium*, since the fungus spores well and Brown (1) has shown that the size of spore inocula, within reasonable limits, has no effect upon subsequent growth. Standard medium plate cultures were prepared from the stock tubes, and kept in an incubator at 20° C. in order that any sign of saltation might be detected. After about three weeks, when spores were forming in abundance, spore masses were transferred from these plates to those of the experimental series.

The carbohydrate series were prepared in the manner described by Seth (13), by replacing the glucose in the standard medium with the kind and quantity of sugar required, and malic acid was used in the acid series. Besides various concentrations of glucose, sucrose, and fructose alone, mixtures of the three approximating in composition to the sugar content<sup>1</sup> of Worcester Pearmain apples obtained from Burwell in 1927, after periods of twenty-five and fifty days in storage at 12° C., were employed. Series containing these mixtures are referred to as *X* and *X'* respectively. The proportions of sugar in each, taken from unpublished data supplied by Dr. Archbold, are as follows:

Series.	Glucose %.	Sucrose %.	Fructose %.
<i>X</i> (Worcester Pearmain apples after 25 days' storage.)	1.5	2.4	7.3
<i>X'</i> (Worcester Pearmain apples after 50 days' storage.)	1.9	1.5	7.6

In the present investigation, series of media of different nitrogen content have also been prepared by varying the concentration of asparagin in the standard medium.

For convenience, the composition of the medium is indicated in the text by the figure representing the percentage concentration of the varying constituent, e.g. 9G represents a medium containing 9 per cent. glucose, 17.1S represents 17.1 per cent. sucrose, 0.25A represents 0.25 per cent. malic acid, and 0.01N represents 0.01 per cent. nitrogen calculated from the weight of asparagin in the medium.

Glucose media were sterilized by autoclaving at 10 lb. pressure for fifteen minutes; media containing sucrose or fructose were steamed for thirty minutes on three consecutive days. Malic acid solutions of the required concentration were autoclaved in separate test-tubes and added

<sup>1</sup> Proportions of sucrose, glucose, and fructose vary considerably with locality and season.

to the bulk of the medium when the latter, after sterilization, had cooled to about 55° C.

Sterilized Petri dishes of uniform size were filled with medium to a depth of (approximately) 0.75 cm., inoculated at the centre with the fungus, and incubated at 20° C. Plates were always prepared in triplicate to increase the reliability of measurements.

The diameter of the culture in each plate was measured along two or more directions, according to the regularity of growth, at intervals usually of three days. Comparisons of growth are based chiefly on length of radius after nine days, since the more rapidly growing cultures reached the margin of the plate in very little over that time.

### III. GENERAL OBSERVATIONS ON GROWTH.

The general morphological characters of the chief fungi used when grown on the standard medium, are tabulated below :

Fungus.	Colour of mycelium.	Nature of mycelium.	Colour of substrate.	Spores.
<i>Pleospora</i> P. 20.	Light to dark grey.	Compact, tufted. Margin somewhat diffuse.	Blue-black to edge of culture.	Very few dark conidia. No ascospores.
<i>Polyopeus purpureus</i> . Poly. 10.	Grey to white.	Compact, short, granular.	Colourless-purple-amber. Almost black at centre.	Hyaline pycnidia. Hyaline spores after 3-4 weeks or no spores at all.
<i>Polyopeus</i> Poly. 2.	White.	Slight, superficial.	None.	Hyaline pycnospores in black, long-necked pycnidia.
<i>Alternaria</i> A. 1.	Dark, olive-grey.	Compact, short, fluffy.	Blue-black almost to edge of culture.	Dark conidia very numerous.
<i>Alternaria tenuis</i> .	Dark, olive-grey.	Flocculent.	Black.	Dark conidia very numerous.
<i>Fusarium fructigenum</i> Fus. A.	White.	Sparse. All but a fringe disappears in time.	Colourless to slightly yellow.	Orange spores in pustules.
<i>F. fructigenum</i> Fus. D.	White.	Fluffy. Blue sclerotia sometimes formed.	Colourless to slightly yellow.	Orange spores often in ring at margin.

Fourteen strains of *Pleospora herbarum* have been studied in less detail than the above. It need only be mentioned here that the mycelium of most strains is fluffy, and varies in colour from white to grey, and that the colour produced in the substrate may be yellow or pink or a mixture of one, or both, of these with black.

The appearance of cultures is altered by variations in acidity. With

increased acid, the substrate colour becomes paler in cultures of *Pleospora* and *Alternaria*, and progressively deepens from yellow to almost brown in *Fusarium* plates. With *Polyopeus purpureus*, the purple colour tends to darken as acid increases from 0.0A to 0.15A, and to become lighter with further addition of acid. *Pleospora* mycelium becomes whiter, *Polyopeus* more fluffy, and *Alternaria* less fluffy with increasing acid, while *Fusarium* remains normal to about 0.3A but is reduced to a wrinkled, non-sporing felt as acid increases to 1.2A. *Fusarium* produces spores in most of the media, *Pleospora* forms a very few conidia in media of low acidity, and *Alternaria* spores very well, even at 1.2A. Rudimentary pycnidia were often formed in *Polyopeus* cultures, but no spores were obtained during the experimental period of nine days.

Variations in sugar content do not greatly affect the appearance of the fungi used in this investigation. The only observable changes were that the substrate colour in plates of *Pleospora* and *Alternaria* becomes paler with increased glucose, and that 9G and 12G serve to prevent the changes in substrate colour which occur in *Polyopeus* cultures with increasing acid at lower glucose concentrations. On 17.1S, *Fusarium fructigenum* A spreads as a very regular, pellucid film on which white aerial mycelium develops later, while on fructose the mycelium is short and granular to the margin. Strain D responds in much the same way, but the margin of growth is extremely irregular when the acid content exceeds 0.05A. The colour produced in the substrate by *Fusarium* is a very pale yellow or is absent on 17.1S, but is a deep yellow to brown on 9F. Sucrose and fructose cultures of the other fungi resemble in appearance, but not in dimensions, the corresponding glucose cultures.

Nitrogen starvation results in a short, sparse growth of *Pleospora*, *Polyopeus*, and *Alternaria*, but at all the experimental concentrations of nitrogen exceeding 0.02N, these fungi are of normal appearance. *Fusarium* develops as a thin film in the absence of nitrogen, becomes thicker and fluffier with moderate increase of this element, but produces a wrinkled felt of mycelium in media containing 0.08N to 0.12N.

More detailed descriptions of the strains of *Fusarium fructigenum* on standard medium, and of the modifications in growth-form which occur with certain changes in composition of medium, may be found in a previous paper by Brown (1).

In the standard medium the fungi spread fairly uniformly, and the margin of the culture is usually well defined. Cultures of *Pleospora* in media where the concentration of both carbohydrate and acid variates is low, are regular, but the margin is so thin that its limits are difficult to determine. At high concentrations of acid, carbohydrate, or nitrogen, all the fungi grow irregularly, and several measurements are necessary to ensure that the mean may be a fair indication of the extent of spread.

Accurate estimates of spread of *Fusarium* presented the greatest difficulty. Some cultures were quite regular, others varied in diameter by as much as 6 mm. along different directions, while discrepancies between replicates and between values obtained on repetition of a series were greater than with the other fungi. No definite sectoring was observed. As a possible means of reducing irregularities, mycelial inoculation, as employed for the other fungi, was tried for *Fusarium* but, as the mycelium, being tough, was difficult to divide into fragments of uniform size, and the resulting growth was no more regular nor consistent than when plates were inoculated with spores, spore inoculation, with its advantage of greater speed, was retained. This irregularity has not been satisfactorily explained.

With the exception of *Fusarium fructigenum* D, which almost ceased to grow after eight days on 1·8G medium, the fungi were not of a strongly-staling type. The difference in behaviour of the strain D and of *Polyopeus* 10 (weakly-staling) on media of low glucose content is shown in Fig. 1.

#### IV. GROWTH IN DIFFERENT COMBINATIONS OF CARBOHYDRATE AND MALIC ACID.

##### (a) *Pleospora*.

Radial spread of P. 20 on series of media in which 2G, 9G, 12G, and the equivalent molecular concentrations of sucrose and fructose (3·8S, 17·1S, 22·8S, 2F, 9F, and 12F) were combined with 0·0A, 0·15A, 0·3A, and 0·6A was first investigated. Three plates of each combination were prepared and the degree of correspondence between replicates, as exemplified by 2G, 9G, and 12G series, is demonstrated in Table I, which gives the mean diameter for each plate nine days after inoculation.

TABLE I.

*Pleospora* P.20. Diameter in Millimetres after Nine Days' Growth on Media varying in Glucose and Malic Acid Content.

Glucose %.	Malic acid %.			
	0·0	0·15	0·3	0·6
2·0	80·0	59·5	46·3	29·0
	83·0	61·0	47·0	28·5
	80·0	61·0	46·5	29·0
9·0	83·0	63·0	43·5	25·0
	88·0	65·0	44·3	25·8
	84·0	63·5	45·0	24·9
12·0	84·0	65·0	41·0	24·0
	86·0	63·0	41·0	24·2
	84·0	63·0	41·0	24·5

The mean radius, calculated from replicates, on the different media after nine days' growth, is given in Table II.

TABLE II.

*Pleospora P.20. Radius in Millimetres after Nine Days' Growth on Media varying in Carbohydrate and Malic Acid Content.*

Carbohydrate.		Malic acid %.			
Nature.	%.	0.0	0.15	0.3	0.6
Glucose	2.0	39.5	29.3	22.4	13.4
	9.0	41.5	30.9	21.3	11.6
	12.0	41.3	30.8	19.5	11.1
Sucrose	3.8	42.5	30.3	22.8	14.0
	17.1	47.0	30.0	21.4	16.0
	22.8	45.0	30.0	19.9	12.5
Fructose	2.0	39.0	30.5	22.4	13.8
	9.0	40.7	29.8	23.9	14.5
	12.0	39.0	31.0	23.0	9.3

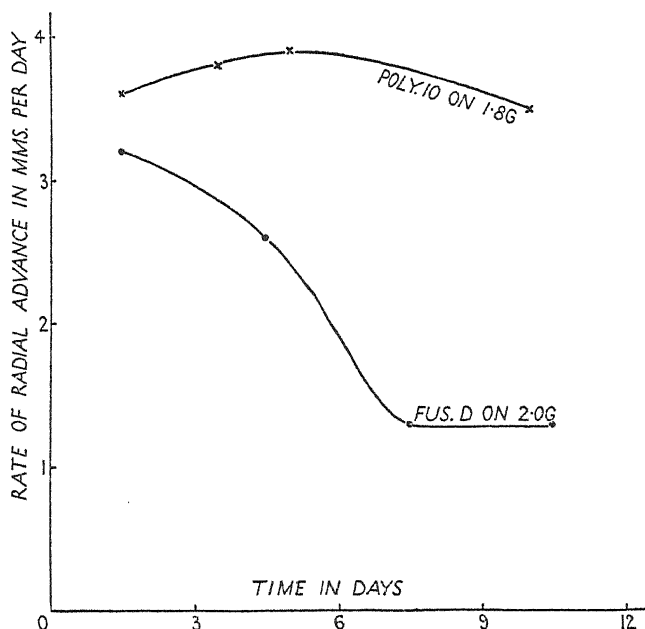


FIG. 1. *Polytopus 10* and *Fusarium fructigenum* D. Comparison of changes in rate of spread associated with increasing age of cultures.

It will be observed that, within the range employed, the nature and concentration of carbohydrate has very little effect. Differences appear to be almost entirely due to variations in acidity, and to obtain more detailed information on this point further series were made containing 12G and 22.8S combined with 0.0, 0.02, 0.04, 0.06, 0.08, 0.10, and 0.15A and 12F combined with 0.00, 0.05, 0.10, 0.15, 0.3, and 0.45A.

The radius after nine days' growth was plotted against acid con-

centration for each carbohydrate, as shown in Fig. 2, from which the following points of interest arise:

1. The curves are all of the same type.
2. With all three sugars radial spread at very low acidities increases

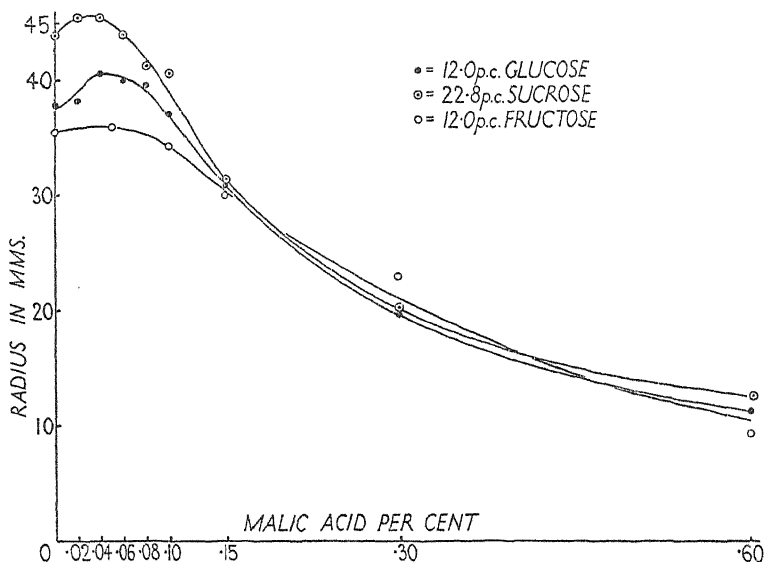


FIG. 2. *Pleospora* 20. Graph showing radial spread in relation to acid concentration at equivalent concentrations of different sugars.

with increasing acidity to a maximum, and then falls off with further addition of acid.

3 The maximum is well marked with sucrose at 0.03A and with glucose at about 0.05A; that for fructose apparently lies at about 0.04A, but is barely perceptible.

4 At low acidities spread is greatest in sucrose, rather less in glucose, and still less in fructose.

5. Spread in all three sugars is equal at 0.15A.

6. The curve for fructose lies slightly above the other two over the range 0.15 to 0.45A.

7. From 0.15 to 0.6A spread steadily decreases by about the same degree in all three sugars.

The fungus was also grown on the series of media *X* and *X'*, which contain combinations of the three sugars.

The curves obtained (see Fig. 3) are of the same type as for single sugars. It should be noted that growth is slightly better in *X* than in *X'* for all the experimental concentrations of acid.

Fourteen strains of *Pleospora herbarum* (P. 1 to P. 16, excepting P. 6 and P. 14) were also grown on the standard medium with 0.0, 0.25, and

0.5A. Although the strains varied considerably in their rate of spread, they were all less active at the higher acidities. The degree of effect produced by acidity differed with different strains and is illustrated in Fig. 4,

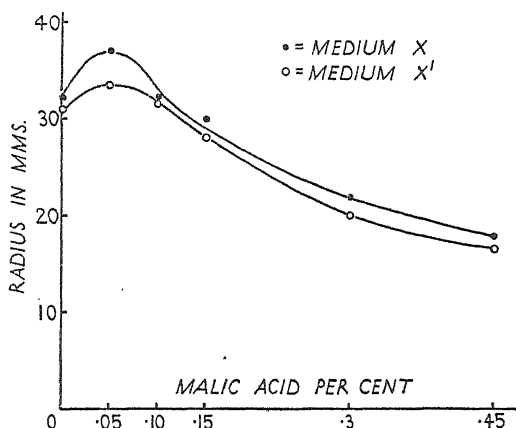


FIG. 5. *Pleospora* 20. Graph showing radial spread in relation to acid concentration in different mixtures of sugars.

where the vertical lines in groups of three for each strain represent radius on media with 0.0, 0.25, and 0.5A respectively.

The two strains P. 4 and P. 9 were also tested on media containing 9G, 12G, and 17.1S combined with 0.0, 0.3, and 0.6A. Table III indicates the relative spread in these media. P. 4 and P. 9 were found to resemble the strain P. 20 discussed in greater detail above, in that they are little affected by character and concentration of carbohydrate within the experimental limits, while their spread is considerably less at 0.3A and still more reduced at 0.6A.

TABLE III.

*Pleospora herbarum* (Strains P. 4 and P. 9). Radius in Millimetres after Nine Days' Growth on Media varying in Carbohydrate and Malic Acid Content.

Strain.	Carbohydrate.		Malic acid %.		
	Nature.	%.	0.0	0.3	0.6
P. 4	Glucose	9.0	34.0	12.5	8.0
		12.0	30.0	11.0	9.0
	Sucrose	17.1	42.0	12.0	9.0
P. 9	Glucose	9.0	42.0	21.0	10.5
		12.0	42.0	18.0	10.5
	Sucrose	17.1	38.0	16.3	10.5

The spread of P. 4, like that of P. 20, appears to be somewhat favoured



by the substitution of sucrose for an equivalent concentration of glucose when no acid is present, but the same change in sugar content slightly retards spread of P. 9.

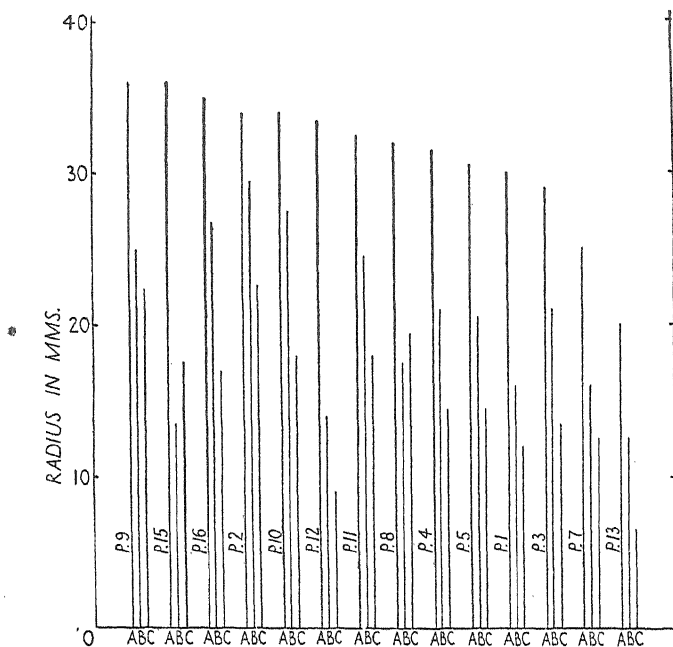


FIG. 4. Diagram showing radial spread of fourteen strains of *Pleospora herbarum* in media with and without acid. (A = 0.0 per cent., B = 0.25 per cent., C = 0.5 per cent. malic acid.)

(b) *Polyopeus*.

The same procedure was followed with Poly. 10 as with P. 20. Data obtained for three concentrations of single sugars with four concentrations of acid are given in Table IV.

TABLE IV.

*Polyopeus purpureus* (Poly.10). Radius in Millimetres after Nine Days' Growth on Media varying in Carbohydrate and Malic Acid Content.

Carbohydrate.		Malic acid %.			
Nature.	%.	0.0	0.15	0.3	0.6
Glucose	2.0	32.3	28.4	19.5	10.0
	9.0	24.3	25.3	17.0	9.3
	12.0	21.5	22.8	15.5	10.0
Sucrose	3.8	33.2	28.3	18.9	10.5
	17.1	21.2	24.9	16.8	10.9
	22.8	19.6	23.3	16.3	10.0
Fructose	2.0	32.9	29.1	20.1	10.8
	9.0	27.5	27.0	17.3	0.0
	12.0	20.8	23.9	16.0	0.0

It should be noted that, as with *Pleospora* 20, radial spread is approximately equal in equivalent concentrations of the three sugars, provided growth occurs at all. Any increase in concentration of carbohydrate, how-

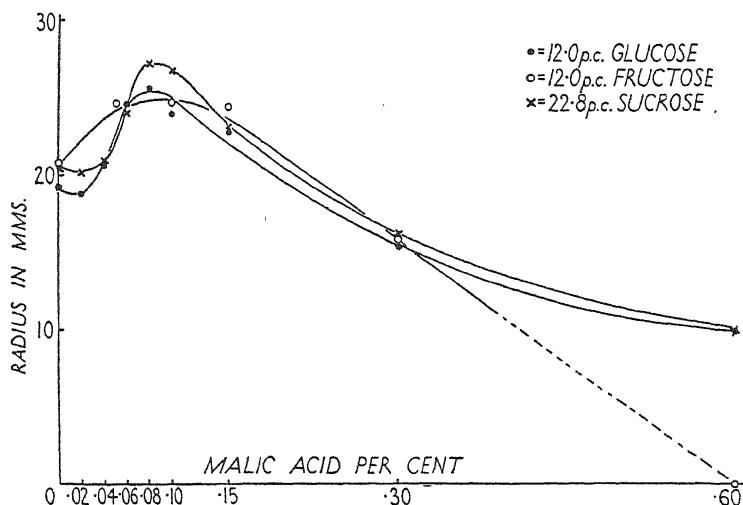


FIG. 5. *Polyoporus* 10. Graph showing radial spread in relation to acid concentration at equivalent concentrations of different sugars.

ever, reduces the rate of spread. The effect decreases with increasing acidity until, at 0.6A, the extent of spread in all the media, with the exception of 9F and 12F, is the same. *Poly.* 10, unlike *P.* 20, is most affected by acid when the carbohydrate constituent is fructose, since no growth takes place in the combinations of 9F and 12F with 0.6A. Graphs illustrating fungal response to acidity in media of 12G, 22.8S, and 12F are given in Fig. 5.

Points to observe are that:

1. The fructose curve resembles the graphs for *P.* 20. The other two differ from these only in the presence of a slight depression of growth as acid is increased from 0.0 to 0.2A.
2. The curves for glucose and sucrose are close together throughout their entire length; that for fructose lies near the others from 0.0 to 0.25A and then falls steeply away to the base line, confirming the view that this fungus is most affected by acid when the carbohydrate present is fructose.
3. From 0.02A radial spread increases with increasing acidity to a maximum value at about 0.09A in each sugar, and becomes progressively less with further addition of acid.

The concentration of acid which *Poly.* 10 can tolerate in conjunction with 12F is somewhat indefinite. It is certainly less than 0.6A since eight inocula on the medium 12F, 0.6A, showed no growth after twelve days,

whereas on a favourable medium growth is apparent after two days. When eight inocula were placed on the medium 12F, 0.3A, five had started

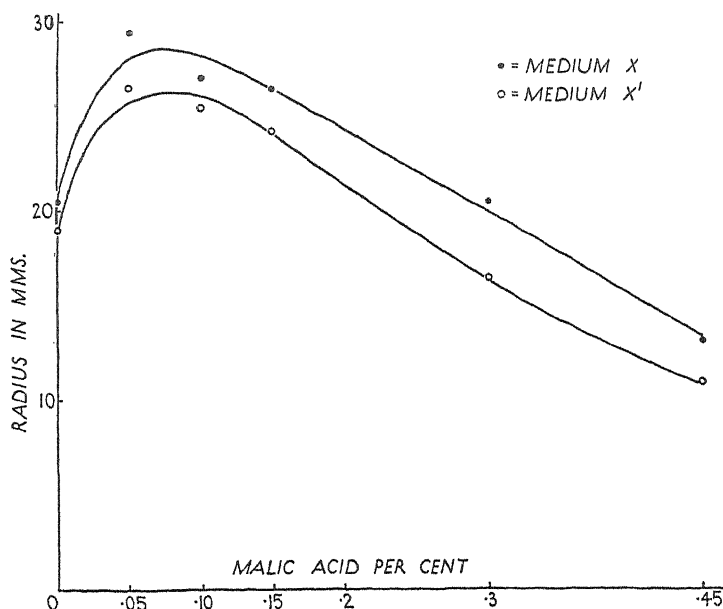


FIG. 6. *Polyopeus* 10. Graph showing radial spread in relation to acid concentration in different mixtures of sugars.

to grow after five days, another started on the eighth, while the other two had not grown on the twelfth day.

Fig. 6 shows spread on media *X* and *X'* in relation to acid.

Growth, as with *P. 20*, is better on the mixture corresponding to the apple after storage for the shorter period. The curves are probably of the same type as those for single sugars, but since the effect of very low acidities was not investigated, it is impossible to state definitely whether the curves fall from 0.02A to zero acid.

TABLE V.

*Polyopeus 2. Radius in Millimetres after Nine Days' Growth on Media varying in Carbohydrate and Malic Acid Content.*

Carbohydrate.		Malic acid %.		
Nature.	%.	0.0	0.3	0.6
Glucose	0.2	20.0	12.3	5.0
	9.0	27.0	4.5	0.0
	12.0	24.0	4.2	2.5
Sucrose	17.1	28.0	4.5	0.0

Another species of *Polyopeus*, *Poly. 2*, was tested on media of acid

content 0.0, 0.3, and 0.6A combined with 0.2G, 9G, 12G, and 17.1S. The results are given in Table V.

This species resembles *Poly. 10* in its general response, but spreads more rapidly than the latter on media without acid. It is also somewhat less tolerant of acid, since 0.3A causes a decrease of spread to about one-fifth of its value in 0.0A and 0.6A further reduces it almost, or quite, to zero when the sugar content is 9G, 12G, or 17.1S.

(c) *Alternaria*.

As before, radial spread in relation to three concentrations of the single sugars combined with four concentrations of acid was first investigated, using *Alternaria*, A.1. The results are embodied in Table VI, and show that radial spread is again little affected by the nature and concentration of the sugar. Extent of spread in media without acid varies very little with increasing fructose, glucose, or sucrose; addition and increase of acid appears to reduce spread by a degree which varies inversely with the quantity of fructose present, hence at 0.3A and 0.6A, growth is considerably greater in 12F than in 2F.

TABLE VI.

*Alternaria* (A. 1). Radius in Millimetres after Nine Days' Growth on Media varying in Carbohydrate and Malic Acid Content.

Carbohydrate.		Malic acid %.			
Nature.	%.	0.0	0.15	0.3	0.6
Glucose	2.0	41.2	30.1	17.4	9.8
	9.0	46.3	32.8	17.3	8.6
	12.0	45.5	31.9	17.9	7.9
Sucrose	3.8	41.5	28.8	16.3	9.0
	17.1	39.9	28.8	17.8	6.6
	22.8	36.3	27.5	18.3	7.6
Fructose	2.0	42.1	31.8	20.0	12.0
	9.0	43.8	37.0	30.0	18.1
	12.0	41.9	37.2	31.6	18.3

Graphs of radial spread in media containing the equivalent sugar concentrations 12G, 22.8S, and 12F, combined with various acidities, are given in Fig. 7.

Here it should be noted that:

1. The curves for glucose and sucrose are similar to those in previous figures, but that for fructose shows no rise to a maximum in the region of low acidity.

2. The peak for glucose is less pronounced than that for sucrose, and has its maximum point at a slightly lower acidity (0.02A as compared with 0.06A for sucrose).

3. The course followed by the curves indicates that radial spread over the range 0.02A to 0.08A is least in fructose, but that at higher acidities it is considerably greater in this sugar than in the others. The intersection

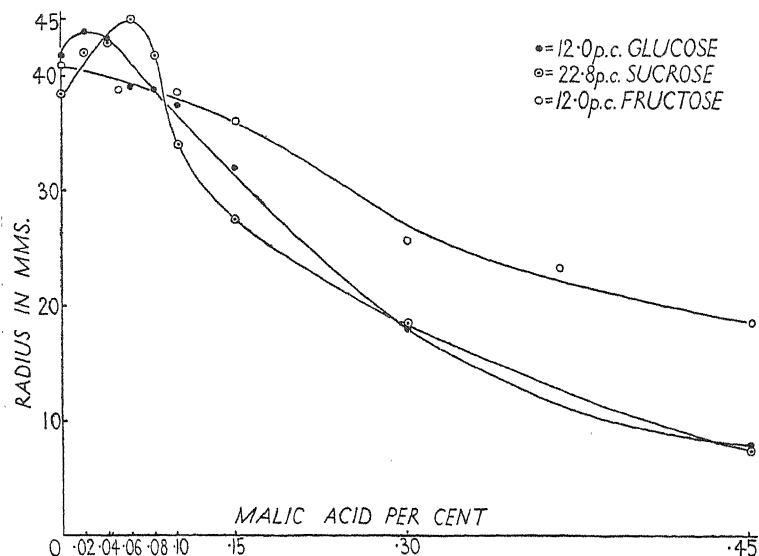


FIG. 7. *Alternaria I.* Graph showing radial spread in relation to acid concentration at equivalent concentrations of different sugars.

of the curves at 0.1A indicates that, at this concentration of acid, kind of sugar has little effect on rate of spread.

Fig. 8 represents spread in the media *X* and *X'*. Fructose predominates in the mixture and the graphs are similar to that obtained for fructose alone. It should be observed that the medium *X*, corresponding to the apple at the earlier stage, again favours spread a little more than the other.

TABLE VII.

*Alternaria tenuis.* Radius in Millimetres after Nine Days' Growth on Media varying in Carbohydrate and Malic Acid Content.

Carbohydrate.		Malic acid %.		
Nature.	%.	0.0	0.3	0.6
Glucose	0.2	27.0	21.0	13.0
	9.0	44.0	20.0	11.0
	12.0	26.0	11.0	10.0
Sucrose	17.1	20.3	8.0	0.0

Another species of *Alternaria*, *A. tenuis*, was also tested on a number of media. The results are given in Table VII, and suggest that this fungus

differs from *Alternaria* A. 1 in being more sensitive to changes in both nature and concentration of carbohydrate. 9G appears to be more conducive of rapid growth than are other concentrations of glucose or the

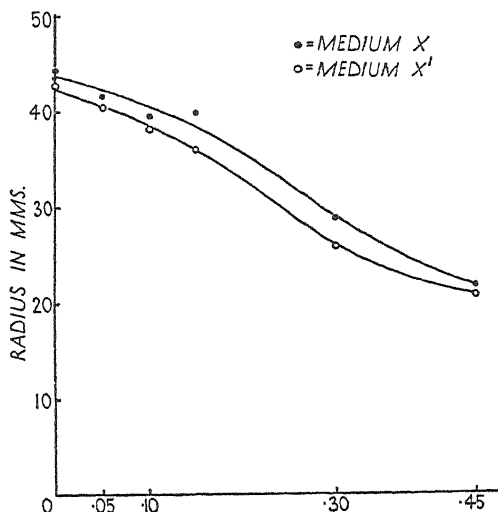


FIG. 8. *Alternaria* 1. Graph showing radial spread in relation to acid concentration in different mixtures of sugars.

equivalent concentration of sucrose. 17.1S is less favourable than any of the concentrations of glucose and supports no growth at all at 0.6A. Acid reduces growth throughout but, as with Poly. 2, its effect is least at the low glucose concentration of 0.2G.

(d) *Fusarium fructigenum*. Strain A.

This fungus was grown on series of media in which every combination of 0.0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, and 1.2A with 1.8G, 2.7G, 4G, 6G, 9G, 12G, 17.1S, 9F, X, and X' was employed.

Radial spread in relation to acidity for four of the glucose concentrations is shown in Fig. 12. The curve for 9G, which appears in Fig. 11, and that for 6G would lie between those given, and are omitted for the sake of clearness in the figure.

It is seen from Fig. 9 that rate of spread falls with increasing acidity. The fall is very rapid over the range 0.05 to 0.1A, and then becomes more gradual. Slight growth was observed at 1.2A.

Fig. 10 illustrates the effect of changing glucose concentration at constant acidities. Growth tends to be abnormal when the acid content is high, and for this reason graphs for concentrations higher than 0.2A are not given. The curve for spread in medium without acid shows a rising limb (1.8 to 4.0G), a maximum point at about 4.0G, and a falling limb

over the range 4.0 to 12.0G. In graphs relating to media which contain acid a rising limb is not well-developed over the range of glucose studied,

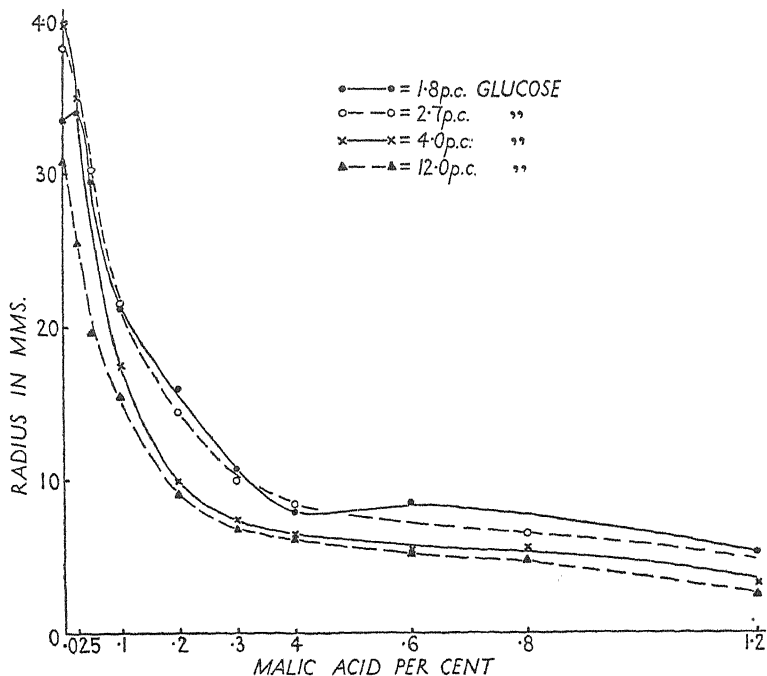


FIG. 9. *F. fructigenum* A. Graph showing radial spread in relation to acid concentration at different concentrations of glucose.

and the maximum is found nearer zero point. At 0.2A the rate of spread undergoes little change over the range 4 to 12G.

The effects of substituting one sugar for an equivalent quantity of another are brought out in Fig. 11, which shows that, while the curve for sucrose resembles that for glucose, glucose favours spread slightly more than sucrose. The reaction to changing acidity in the fructose medium differs remarkably from any yet mentioned. From 0.0A to 0.2A the fructose graph takes a straight course near the curves for the other sugars but sloping less steeply, then it falls suddenly to the base line showing that the maximum acid content which can be tolerated by the fungus on 9F is very low, and that the difference in concentration between an acidity on which good growth occurs and one supporting no growth at all is slight.

Radial spread of strain A on the mixtures X and X' is represented in Fig. 12, and is seen to be chiefly influenced by the fructose content of the media. The difference in rate of spread in the two media is negligible. The exact concentration of acid which just permits of growth is uncertain since at 0.3A and 0.4A growth occurred on some plates but not on others.

(e) *F. fructigenum*. Strain D.

Strain D was grown on the series of media described for strain A. Its response in media differing in glucose and acid content is illustrated in Fig. 13.

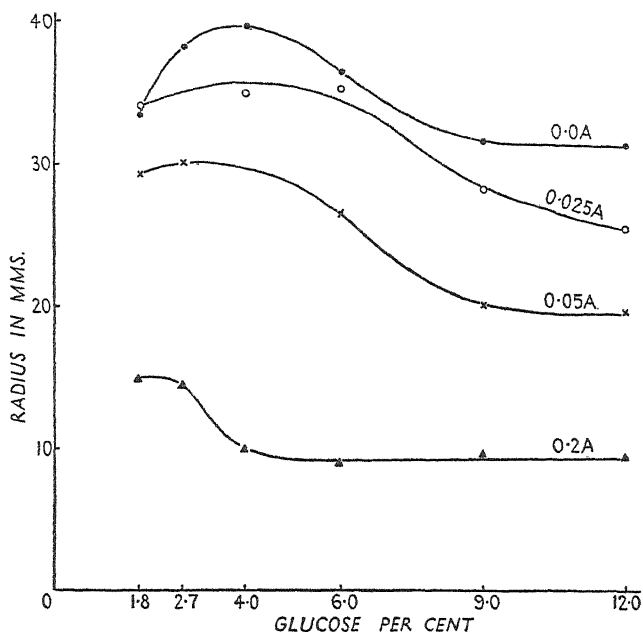


FIG. 10. *F. fructigenum* A. Graph showing radial spread in relation to glucose concentration at different concentrations of acid.

It may be observed that :

1. The curves for 6G and 9G resemble those obtained for *Fusarium* A.
2. The curves for low glucose concentrations (1.8G and 2.7G) resemble some of the curves given in Figs. 2, 5, and 7 in rising to a peak, but the maximum value lies at the lower acidity of 0.05A.
3. Radial spread rapidly falls as acid content increases from 0.05A to 0.25A, but little change occurs with further increase in acid.

Comparison of Figs. 9 and 13 shows that, at low acidities, strain D makes much less rapid progress than strain A, but is less affected by increasing acid and, at high acidities, the two fungi spread at equal rates.

The response of strain D to changes in the concentration of glucose at constant acidities is illustrated in Fig. 14. The curves resemble those for strain A in general nature, except that the curve for 0.0A bends upwards over the range 9G to 12G. The increase in spread recorded at 12.0G is only 2 mm. in the nine days, but a similar increase has been recorded on



five different occasions. The curve for 0.025A closely resembles that obtained for 0.0A with strain A (Fig. 10).

Fig. 15 represents radial spread in relation to kind of sugar. The

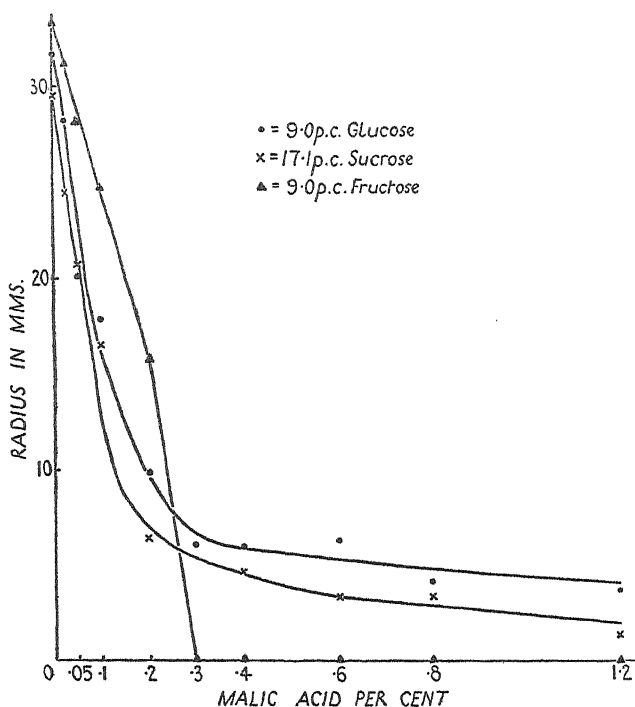


FIG. 11. *F. fructigenum* A. Graph showing radial spread in relation to acid concentration at equivalent concentrations of different sugars.

curves show that sucrose favours radial spread more than glucose or fructose when acid is absent. When acid is present strain D resembles strain A in spreading to nearly the same extent in media containing glucose and sucrose respectively. As with *Alternaria* (Fig. 7), fructose favours spread more than glucose or sucrose when the level of acid exceeds 0.1A approximately.

Graphs representing radial spread in the series *X* and *X'* are given in Fig. 16. The curves are of distinctive appearance. At low levels of acid they resemble the glucose and sucrose curves, but at higher levels the fructose curve given in Fig. 15. This result indicates that rate of spread for levels of acid exceeding 0.1A is chiefly determined by the fructose constituent.

*F. fructigenum* D differs from *Pleospora* 20, *Polyopeus* 10, and *Alternaria* 1 in spreading in nine days, slightly more in *X'* than in *X*, except over the range 0.2A to 0.4A.

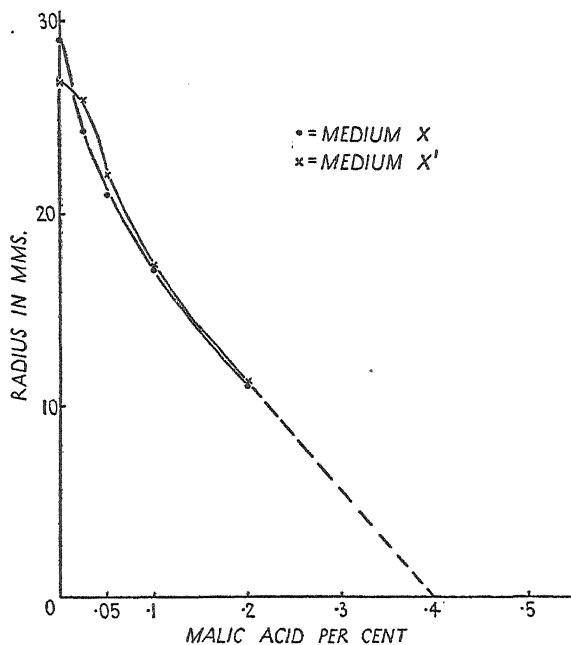


FIG. 12. *F. fructigenum* A. Graph showing radial spread in relation to acid concentration in different mixtures of sugars.

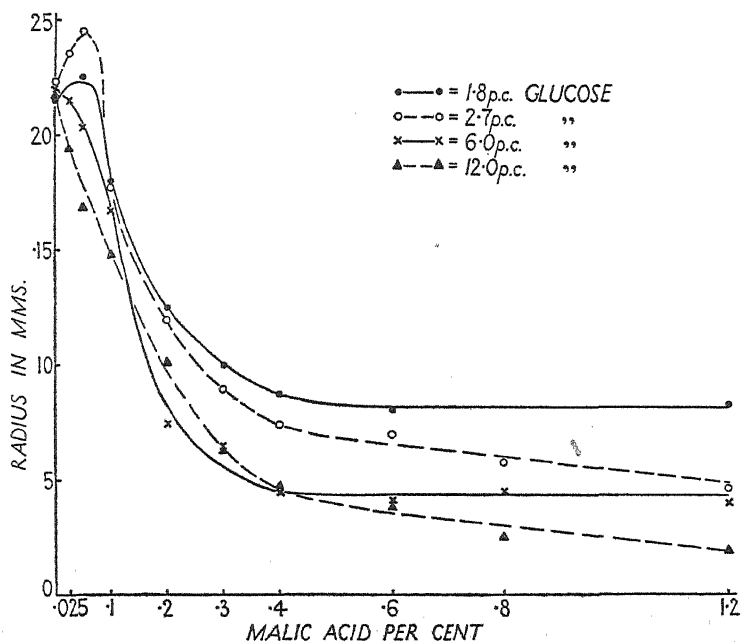


FIG. 13. *F. fructigenum* D. Graph showing radial spread in relation to acid concentration at different concentrations of glucose.

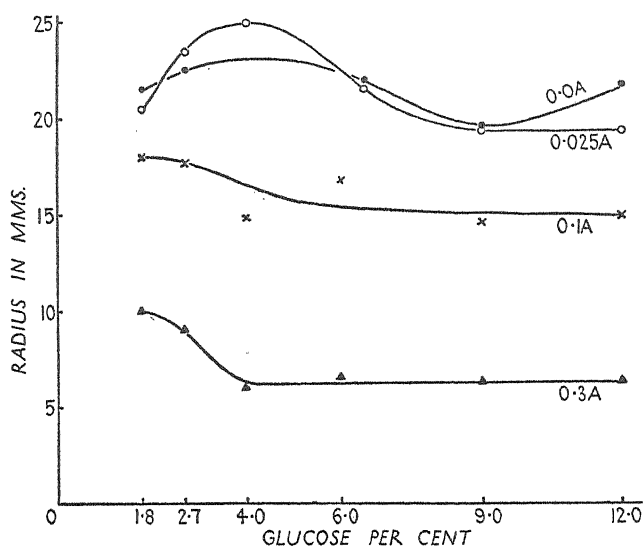


FIG. 14. *F. fructigenum* D. Graph showing radial spread in relation to glucose concentration at different concentrations of acid.

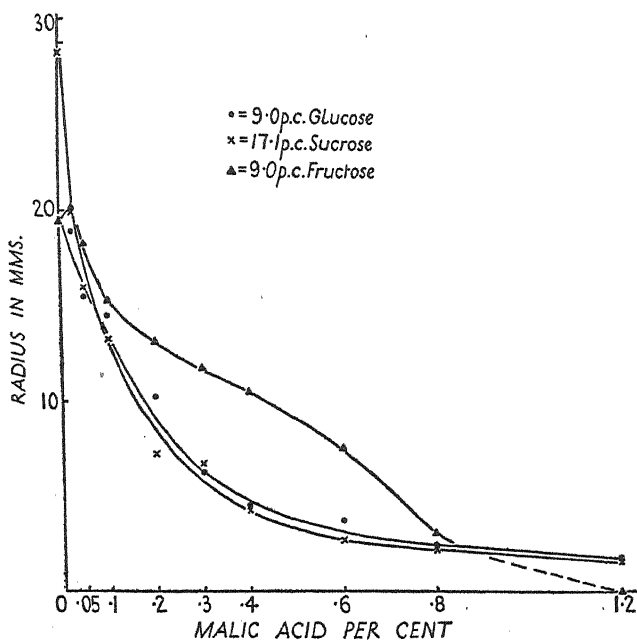


FIG. 15. *F. fructigenum* D. Graph showing radial spread in relation to acid concentration at equivalent concentrations of different sugars.

The upper limit of acid concentration which can be tolerated is again indefinite, since growth occurred in some but not all of the plates at 0.8A and 1.2A.

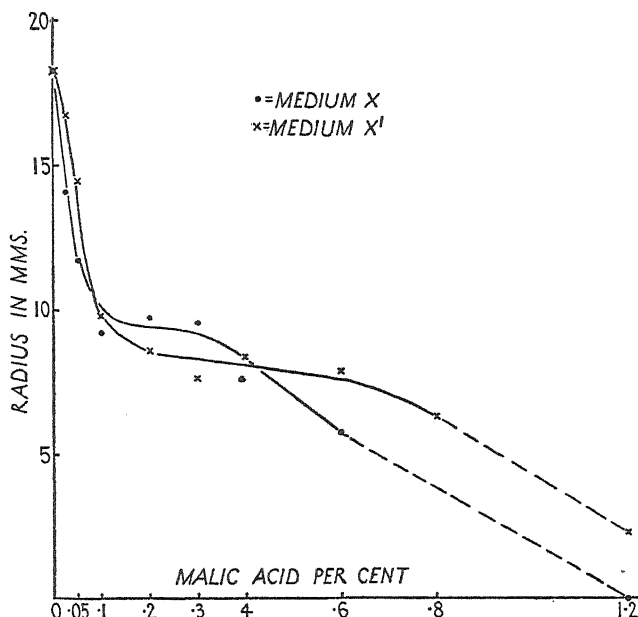


FIG. 16. *F. fructigenum* D. Graph showing radial spread in relation to acid concentration in different mixtures of sugars.

## V. GROWTH IN DIFFERENT COMBINATIONS OF ASPARAGIN AND MALIC ACID.

(a) *Pleospora* 20, *Polyopeus* 10, and *Alternaria* 1.

These three fungi were grown on series of media containing 12G in conjunction with every combination of 0.0A and 0.15A with ten concentrations of asparagin, introducing nitrogen in the proportions 0.0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08, 0.10, and 0.12 per cent. Fig. 17 A and B illustrate the results in 0.0A and 0.15A respectively. The curves for *P.* 20 and *Poly.* 10 are based on measurements on the ninth day after inoculation. The curve for *Alternaria* in Fig. 17 A represents radial spread on the sixth day, since the plates were filled by the ninth day; those given for the same fungus in Fig. 17 B illustrate spread recorded on both occasions.

It will be noted that the presence of acid in the medium affects the fungal response to increasing nitrogen.

The curves given in Fig. 17 A resemble one another fairly closely, and suggest that variations in nitrogen content produce little effect in the absence of acid. The curves vary as regards the point at which maximum spread occurs, viz. 0.06N for *Pleospora*, 0.07N for *Polyopeus*, and 0.04N for

*Alternaria*. Fig. 17 B shows that the three fungi respond to addition of 0.15A to the nitrogen series of media in different ways. Radial spread of Poly. 10 consistently increases with increasing nitrogen, the effect being

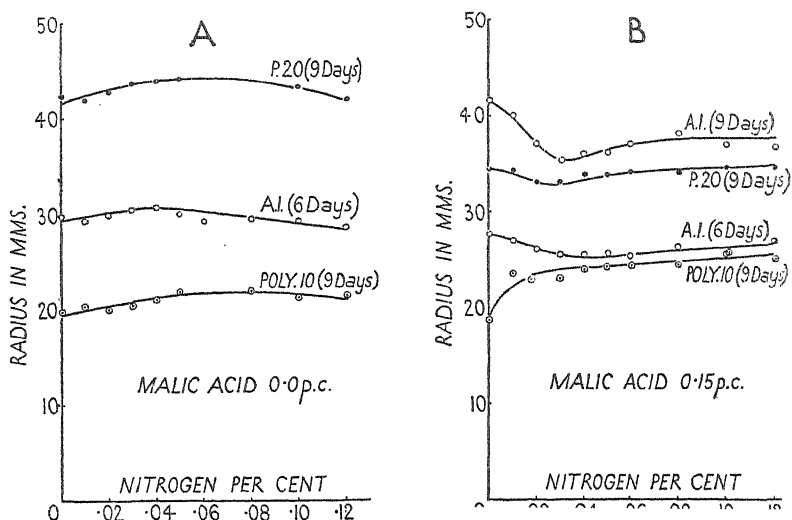


FIG. 17. *Pleospora 20*, *Polyoporus 10*, and *Alternaria 1*. Graphs showing radial spread in relation to nitrogen concentration. A, in media without acid; B, in media with acid.

most marked over the range 0.0N to 0.02N. Spread of A. 1 and P. 20 diminishes over the range 0.0N to 0.03N, but is favoured by further addition of nitrogen. Changes in the rate of spread of P. 20 are very small.

(b) *F. fructigenum*. Strain A.

Strain A was grown on the ten concentrations of asparagin mentioned for the other fungi, combined with 0.0A, 0.15A, and 0.3A, and a carbohydrate content of 12G. The results are expressed graphically in Fig. 18, A and B.

The curves constructed from data obtained on the twelfth day show a considerable development of tendencies observed on the sixth day. With no acid present in the medium, radial spread increases from near zero point to a maximum at 0.04N, and then diminishes over the range 0.04N approximately to 0.12N. Measurements recorded for six and twelve days respectively show that the rate of spread over the range 0.04N to 0.12N is not uniform; thus at 0.12N the radius in six days is about 20.25 mm.; in twelve days only 30.25 mm., suggesting that a certain amount of staling had taken place. The rate of spread is greatly retarded by adding 0.15A to the medium, as is shown by the distance separating the curves for 0.0A and 0.15A. The maximum point is found nearer zero at 0.01N, and a minimum point is evident at 0.03N. From this point the rate of spread gradually increases over the range 0.03N to 0.10N approximately. When

acidity is increased to 0.30A, the curve rises steadily from near zero point to 0.10N approximately. The change in slope of the curve associated with addition of acid suggests that acidity tends to retard rate of spread

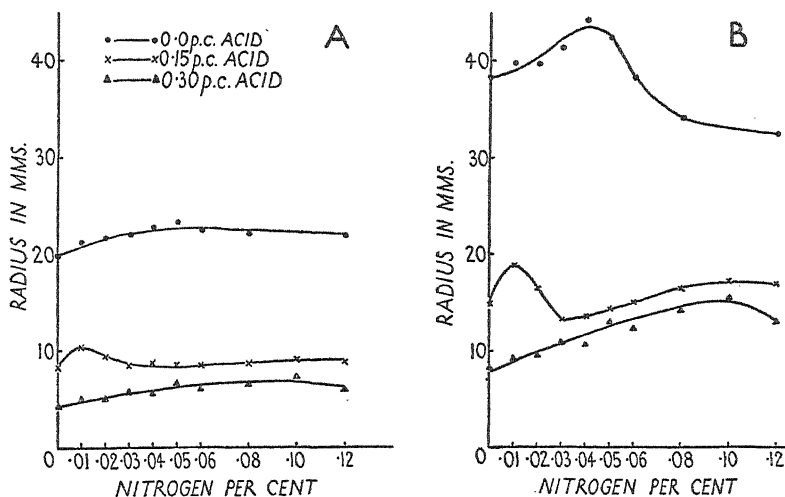


FIG. 18. *F. fructigenum* A. Graphs showing radial spread in relation to nitrogen concentration in media with and without acid. A, after 6 days; B, after 12 days.

more and more as the nitrogen content of the medium falls. The steeper inclination of the curves for 0.15A and 0.30A observed on the twelfth day as compared with that shown on the sixth day suggests that perhaps as time goes on a certain amount of acid is neutralized by alkaline waste products resulting from the metabolic activity of the fungus in the higher concentrations of nitrogen and that the concentration of acid is correspondingly lowered, thereby favouring radial spread.

#### (c) *F. fructigenum*. Strain D.

The series of media described for strain A were also used for strain D. The results are illustrated in Fig. 19, which represents radial spread after nine days. When the cultures on media without acid were examined on the sixth day, only small differences between individual members of the series were observed, but 0.01N and 0.02N appeared to favour spread more than the remaining concentrations of nitrogen. No appreciable staling was apparent. On the ninth and twelfth days strong staling was recorded for all concentrations except 0.01N and the medium without added nitrogen. The form of the curve (Fig. 19) for the series without acid, which shows a sharp fall between 0.01 and 0.02N, may be mainly due to the strongly staling character of the strain D. The curve for 0.15A closely resembles the one obtained when acid is absent, but the shape cannot be explained by staling influences since no staling was recorded in media

containing 0.15A in combination with 0.04N on the ninth day. The curve for 0.3A agrees fairly closely with that given for strain A (Fig. 18 B), the rate of spread increasing with increasing nitrogen content of medium.

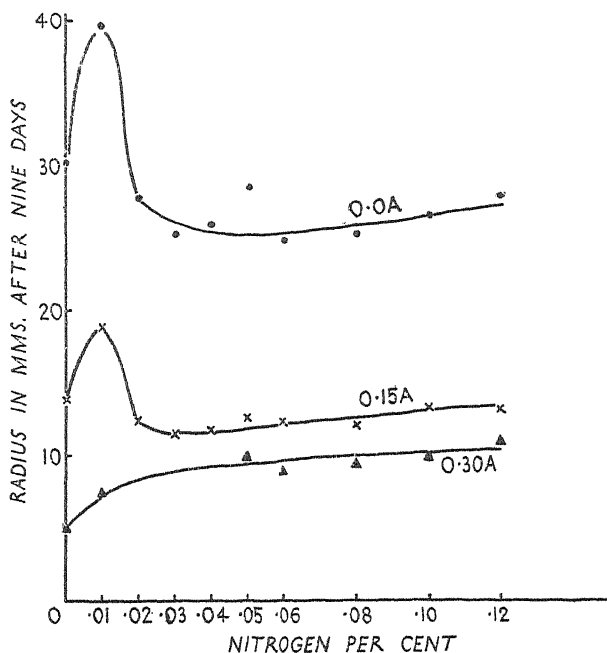


FIG. 19. *F. fructigenum* D. Graph showing radial spread in relation to nitrogen concentration in media with and without acid.

## VI. DISCUSSION.

### *Salient features of the results.*

The curves obtained as a result of plotting radial spread against acid concentration conform to Types I and II recorded by Horne (9) and Seth (13), namely, hyperbolic curves (Type I) shown by *F. fructigenum*, strain A (Figs. 9 and 11); and curves with a rising limb, a maximum point, and a falling limb, as shown by *Pleospora herbarum* (Fig. 2) and *F. fructigenum*, strain D (Fig. 13). After the maximum the curves resemble those of Type I. In curves of Type II the maximum point is found at low concentrations of acid, thus with *Pleospora herbarum* at 0.04A; *Alternaria* at 0.06A; and *Polyopeus purpureus* at 0.8A. With *Polyopeus purpureus* curves 12G and 22.8S show a well-developed maximum and resemble curves 1.4G and 2.75G given by Seth for *C. ludibunda* CC<sub>2</sub>, but with the remaining fungi the maximum point is situated only a few millimetres above the level found near zero acid. The results show, therefore, that radial spread

is inversely related to acid concentration, but the range over which this effect is seen varies somewhat with fungal strain since the presence of a small quantity of acid in the medium favours spread of certain strains.

The fungi under consideration, as far as response to varied acid is concerned, fall between the less active groups 3 and 5 of Horne (9) and Seth (13), whose grouping of species of *Cytosporina*, *Phomopsis*, and *Diaporthe* was based on rate of spread in acid series made up to contain glucose, fructose, and sucrose in proportions found in Cox's Orange Pippin apples. *Pleospora herbarum* (P. 20), *Polyopeus*, and *Alternaria* spread radially only 1 cm. or less in nine days at approximately 0.6A. The strains of *F. fructigenum*, in media containing 12 per cent. glucose combined with 0.4A, spread to about 6 mm. (strain A) and about 5 mm. (strain D) as compared with more than 3 cm. and more than 2 cm. recorded for the respective strains in similar media without acid.

The effect of varying concentration of carbohydrate has not received the detailed consideration given by Seth. As a rule only three (2, 9, and 12G) and exceptionally six (*F. fructigenum*) concentrations of sugar were used, and none exceeded 12G or an equivalent concentration of mixed sugars (glucose, fructose, and sucrose). Within these limits the effect on spread of varying concentrations was not very great. The species and strains of certain species tested differ to a certain extent in their response to varied carbohydrate concentration in media both with and without acid. With *Pleospora* 20 the rate of spread in media without acid is nearly the same at 2, 9, and 12G, but when acid is added (0.3 and 0.6A) the rate tends to fall from approximately 9G. With *Polyopeus* 10 radius of spread in media without acid decreases from 32.3 mm. at 2G to 21.5 mm. at 12G, but the decrease is less marked when acid is present. *Alternaria* 1 spreads more quickly in media containing no acid or weak acid (0.15A) when 9 or 12G is present, but the rate of spread tends to fall with increasing glucose as the level of acid is raised. With *F. fructigenum* (acid absent) spread reaches its maximum development at 4.0G and then diminishes. As more and more acid is added the maximum point is found nearer zero glucose. The curve for 0.2A falls as far as 4.0G, and then remains nearly horizontal over the remaining portion of the range of glucose concentration tested.

The substitution of sucrose for glucose in series yielded no results of importance since only small differences in amount of spread were observed. With some strains sucrose, with others glucose, slightly favoured spread. The most marked effect was obtained with *F. fructigenum* D in the absence of acid, the rate of spread being much greater in the sucrose medium, but with addition of acid the rate declined and corresponded very closely with the rate observed in the glucose media.

The substitution of fructose for glucose produced the following results:

- (1) *Pleospora* 20, rate of spread closely corresponds with that observed



when glucose is present; (2) *F. fructigenum* D and *Alternaria* 1, fructose favours spread at nearly all levels of acid tested as recorded for *P. citri*, Jaffa 18, by Seth (13); (3) *F. fructigenum* A and *Polyopeus* 10, fructose favours spread at relatively low levels of acid, but at higher levels spread is retarded, cf. *C. ludibunda* CE and CA<sub>4</sub> investigated by Seth (13). With *F. fructigenum* A, no growth was observed in media containing 0.3A combined with 9.0F as compared with 6 mm. radial spread recorded for an equally acid medium containing 9.0G instead of fructose.

The curves obtained for spread in mixtures of sugars, as might be supposed from the proportions of the three sugars present, are very similar to the fructose curves and differ from them only in such ways as might be expected from the presence of the other sugars. Those for *Pleospora* 20, for instance, in *X* and *X'* are of the same nature as that for fructose alone, but the rise to a maximum at 0.05A is more marked and approximates to that shown in the glucose or sucrose curves. Again at acidities of about 0.3A the rate of spread of *F. fructigenum* D in *X* is intermediate between the rate in media containing fructose and the rate in media containing glucose or sucrose. With the exception of *F. fructigenum* A, which spreads to the same extent in both mixtures, and *F. fructigenum* D, which tends to spread better in *X'* than in *X* at high or very low acidities, the fungi spread more rapidly in *X* than in the other mixture.

So far the discussion has been confined to reviewing the results obtained with two main variables, viz. acid and sugar. The inclusion of a third variable, nitrogen, renders the problem of interpretation much more difficult, especially when it is remembered that the species under investigation show a staling tendency. Since it was possible to undertake only a limited number of experiments with nitrogen, supplied in the form of asparagin, it is impossible to explain all the results satisfactorily. With *Pleospora* 20, *Polyopeus* 10, and *Alternaria* 1, fungi which show only slight staling in standard medium cultures, increasing nitrogen supply up to approximately 0.12N, in the absence of acid, does not appreciably affect the rate of spread, since, within the experimental range of nitrogen concentration, the difference in radius between cultures on extremes never exceeded 2 mm. A slight tendency to rise to a maximum at some level of nitrogen is evident (Fig. 17 A), and staling does not appreciably increase with increasing nitrogen. The addition of weak acid (0.15A) produced an increase in rate of spread of *Polyopeus* 10 in media containing 0.01N or more, but had no effect in the absence of asparagin. The curve obtained for *Pleospora* 20 is nearly horizontal, possibly with a minimum at 0.03N instead of the maximum at about 0.06N observed when acid is absent. Spread of *Alternaria* 1, for unknown reasons, has its highest value at zero nitrogen and falls to a fairly definite minimum at about 0.03N. Comparison of the curves in Fig. 17 A with those in Fig. 17 B confirms the evidence already

presented (Figs. 2, 5, and 7) that Poly. 10 grows better in 0.15A than in 0.0A while P. 20 and A. 1 are adversely affected by 0.15A.

Turning to *F. fructigenum*, the strains D and A, as was originally demonstrated by Brown (1), are respectively strong and moderately staling strains. The steep gradients shown by the ascending and descending limbs of the curve for strain D in media without acid (Fig. 19) are probably an expression of the strongly-staling character of this strain. It is interesting to note that on the sixth day, only small differences in radial spread between individual members of the nitrogen series were observed, indicating that in early stages of growth, spread is not appreciably affected by altering the concentration of nitrogen. On the ninth day, however, strong staling was recorded for all members of the series except 0.01N and the medium without added nitrogen. Addition of acid should cause the maximum point shown in Fig. 19 to be found further from zero nitrogen, since the acid would neutralize, to a certain extent, the alkaline waste products of fungal metabolism. The system of curves was probably not explored in sufficient detail to bring out this point since the curve for 0.15A, in common with that for acid absent, shows a maximum at 0.01N. In spite of the similarity between the curves, the form was probably determined by different factors since no staling was recorded on the ninth day in media containing 0.15A in combination with 0.04N. With further addition of acid (0.3A) the maximum vanishes and rate of spread is directly related to nitrogen content of medium.

#### *Results considered in relation to advance of fungi within the host tissues.*

Seth (13) found that fungi vary considerably in their response to altered concentration of acid and that their response to acid is in general related to their power of attacking apples, as determined by Das Gupta (2, 3). The fungi isolated from Worcester Pearmain apples, a variety with relatively low acidity (0.4A approximately, falling to zero acid with increasing age), includes, as reported by Horne (10) fungi such as *Botrytis*, *Phomopsis*, &c., which cause fairly rapid decay in both sweet and sour varieties; others such as *F. fructigenum* which cause decay in sweet varieties and may cause spotting in sour varieties; and others such as *Pleospora*, *Polyopeus*, and *Alternaria* which are chiefly responsible for spotting in sweet varieties. According to Seth (13) *P. coneglanensis* and according to Horne (11) *Botrytis* can maintain growth in media containing more than 1.0A. The results presented in this paper show that *F. fructigenum* D is less tolerant of acid, but a certain amount of growth was observed in media containing more than 0.8A. *Pleospora*, *Polyopeus*, and *Alternaria* are, however, unable to maintain growth very well at levels of acidity higher than 0.4A or 0.6A.

When a sweet variety such as Cox's Orange Pippin (0.6A falling to 0.2A approximately) is held in storage under low temperature conditions (1–5° C.) these different fungi attack in sequence, as shown by Horne (11). Thus *Botrytis* attacks early in the storage period, *F. fructigenum* D later, and, later still, *Polyopeus* and *Pleospora*. At high temperatures (15–20° C.) chemical changes take place rapidly in the apple, one fungus rapidly succeeds another, and within a fortnight spots and decayed areas due to all the fungi mentioned may be present, as recorded by Horne for the Worcester Pearmain apples already mentioned.

The reason why spotting is much more prevalent in sweet varieties is because the number of attacking fungi is not limited by acidity. Spotting is of course liable to occur in sour varieties such as Lane's Prince Albert and Bramley's Seedling, but to no appreciable extent, unless acidity is abnormally low, or it is late in the storage season, when acidity has fallen to a minimum. Weak strains, for example *Pleospora*, *Polyopeus*, and *Alternaria*, are rarely responsible for such spots. Horne (7) on one occasion isolated *C. ludibunda* from spots or areas occurring on more than fifty Lane's Prince Albert apples. Horne (11) also found that certain strains of *F. fructigenum* were able to attack Bramley's Seedling apples (1.0A falling to 0.5A approximately), but the rate of invasion was very low compared with that determined for the same fungi in the Cox's Orange Pippin variety.

Changes in chemical composition associated with increasing age of fruit are not confined to acidity which falls to lower and lower levels as time passes. The sugar content also changes both as regards total sugar content and relative proportions of glucose, sucrose, and fructose present in the fruit. These changes result in a final lowering of the total sugar content. The results presented here show that variations in sugar supply affect rate of spread to nothing like the same extent as do variations in acidity. This statement is borne out by the results of the experiments with mixtures of sugars. As observed before (see Figs. 3, 6, and 8), spread, in a mixture (*X*) of glucose, sucrose, and fructose in the proportions in which they are present in Worcester Pearmain apples twenty-five days after gathering, exceeds that in a mixture (*X'*) representing the same fruit twenty-five days later, for all corresponding acidities. When, however, spread in *X* at 0.25A, the concentration of acid normally present in Worcester Pearmain apples after twenty-five days' storage, is compared with spread in *X'* at 0.15A, which represents the concentration of acid in the apple after fifty days' storage, rate of spread in the latter medium is much greater, indicating that fungal response to the fall in acidity is more than sufficient to counteract the slightly adverse effects of the change in sugar composition.

*F. fructigenum* is a somewhat more active parasite than the other species of fungi considered here, and more detailed knowledge of its attacking power is available. Horne (7) has shown that the rate of

invasion of Cox's Orange Pippin by strains A and D increases with age of apple for some weeks after gathering. The acid content of these apples would fall from about 0.7A to (approximately) 0.3A during the storage period. The results described here have shown that such a change in acid concentration has a favourable effect upon the growth of these strains in synthetic media, hence, it would appear that acidity of the fruit is of great importance in determining the extent of attack by *F. fructigenum*. The behaviour of the strains of *Fusarium* on *X* and *X'* also suggests that, as with the other fungi, acidity rather than changes in sugar content of Worcester Pearmain apples will be responsible for any increased susceptibility of the fruit with age.

Horne and Gregory (11) found that the strains of *F. fructigenum* could be arranged in a definite order of attacking power which was independent of variety of apple. Strain D occupied the first position in this series, the weaker strain A an intermediate position. These strains received more detailed attention by Horne in a later paper (7). Samples of Cox's Orange Pippin apples were inoculated every week with strain A on one side and strain D on the other side of individual apples. By plotting radial advance against time, curves were obtained showing progress of invasion by the two strains. Early in the experimental period D proved much more active than A, but as time went on the curves converged until at the end of the experiment the rate of invasion was nearly the same in both cases. During the course of the experiment the acidity of the apples had been gradually falling, probably from about 0.6A to 0.2A. Horne later obtained a similar result, as yet unpublished, with Worcester Pearmain apples in which acidity may fall to zero at the end of the storage period. This varying response may be explained to a certain extent by differences in acidity of the apples. In media containing little or no acid, and therefore comparable with Worcester Pearmain apples in this respect, strain A grows much faster than strain D; but in media containing as much as 0.3A, the two strains grow at approximately the same rate. Here fructose appears to be an important factor, since at higher acidities when fructose is present (Figs. 11, 12, 15, and 16) strain D is more active than strain A.

Nitrogen content of fruit may also influence the degree to which different samples are subject to spotting. Horne and Gregory (11) have shown that a positive correlation (0.737) exists between the rate of advance of *C. ludibunda* and the value of nitrogen concentration in Bramley's Seedling apples, but they point out that the comparisons made were too few to establish the significance of this correlation. Experiments by Horne (7) in which *F. fructigenum* D has been tested on Cox's Orange Pippin have proved that the rate of advance of the fungus in any one year varies directly with the nitrogen content of the fruit, the acid concentration being between 0.3 and 0.4 per cent. This is in agreement with the results

presented in this paper (see Fig. 19), where the growth of strain D is found to be favoured by increasing the nitrogen content of the medium at 0.3A. Recent work by Horne (9) supplies further evidence of a direct relationship between nitrogen content and susceptibility of apples. Newton Wonder apples from some trees which had been ringed and from others not ringed were inoculated with *C. ludibunda* CE, and *Diaporthe perniciosa* DHF. The proportion of nitrogen in the fruit was also estimated. It was found that the apples from the ringed trees contained less nitrogen than the others and were less susceptible to fungal attack (9, p. 285). Again, Bramley's Seedling apples from trees which had received different manurial treatment were inoculated with *C. ludibunda* CE. The fungus was found to advance most rapidly in those apples which by chemical analysis were known to contain the highest proportions of nitrogen (9, p. 288).

The results obtained with the *Fusarium* strains by the present author suggest that although a positive correlation between the nitrogen content and fungal attack may hold good for apples which contain 0.3 per cent. or more of acid, it is possible that the susceptibility of sweet varieties such as Worcester Pearmain may, under certain conditions, be inversely related to the nitrogen content.

No information is available as to the rate of invasion of fruit by *Pleospora*, *Polyopeus*, or *Alternaria* in relation to nitrogen content. The present results suggest that, as far as these fungi are concerned, nitrogen is not as important a factor as acid. Comparison of Figs. 17 A and B with Figs. 2, 5, and 7 shows that the changes in nitrogen concentration have far less effect upon growth than have variations in acidity. The three fungi also respond to changes in nitrogen supply in different ways, hence no generalizations can be made concerning the possible effect of this factor upon the occurrence of spotting.

## VII. SUMMARY.

The chemical factors affecting advance within the host tissues of *Pleospora herbarum*, *Polyopeus*, *Alternaria*, and *Fusarium fructigenum*, fungi responsible for spotting of apples, have been investigated, using the cultural method adopted by Seth.

The curves obtained by plotting radial spread against acid concentration conform to types I and II recorded by Seth, namely, hyperbolic curves, as shown by *F. fructigenum* A; and curves with a rising limb, a maximum point (found at approximately 0.1A), and a falling limb, as shown by *Pleospora*, *Polyopeus*, *Alternaria*, and *F. fructigenum* D (high glucose). These fungi spread very slowly in media containing more than 0.4A, a fact which probably explains why fungal spotting is much more prevalent in dessert (sweet) than in sour varieties of apple.

Changes in the concentration of sugar within the experimental limits did not greatly affect the rate of spread observed at different levels of acid, and little effect was apparent when sucrose was substituted for glucose. The substitution of fructose for glucose yielded the following results: (1) *Pleospora*, rate of spread closely corresponds with that observed when glucose is present; (2) *F. fructigenum* D and *Alternaria*, fructose favours spread at nearly all levels of acid tested; (3) *F. fructigenum* A, fructose favours spread at relatively low levels of acid, but at higher levels spread is retarded. No growth was observed in media containing 0.3A combined with 9.0F. It is therefore inferred that fructose content is partly responsible for the change in the order of attacking power of *F. fructigenum* strains A and D previously recorded as associated with increasing age of fruit (7).

The results further show that the fungi studied vary in response to changes in nitrogen supply. When no acid is present in the media, curves expressing the relationship between radial spread in nine days and nitrogen content, tend to rise to a maximum at a nitrogen concentration little more than 0.0 per cent., and then fall as concentration increases. When acid is present the same fungi vary more widely in response. Thus when 1.5A is added, the curve for *Alternaria* tends to fall as nitrogen concentration increases from zero to about 0.4 per cent., and then pursues a nearly horizontal course. The curves for *F. fructigenum* A and D, on the other hand, rise to a maximum and then fall. With further increase in acid (0.3A) the curves for the strains of *F. fructigenum* rise continuously as nitrogen concentration increases from zero to the relatively high value of about 0.1N. Hence with this fungus a change from 0.15 to 0.3 per cent. in acid content of medium, results in a reversal of the relationship between radial spread and nitrogen concentration for values ranging from 0.01N to 0.03N. This point is of particular interest because it suggests that in apples of low acid content, the usual relationship between radial advance of the fungus and nitrogen content of the fruit may possibly be reversed.

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# The Absorption and Accumulation of Solutes by Living Plant Cells.

## VI. The Absorption of Potassium Bromide from Dilute Solution by Tissue from Various Plant Storage Organs.

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With one Diagram in the Text.

THE absorption of solutes by living cells has attracted considerable attention, no doubt because it involves problems equally perplexing from the physico-chemical and biological points of view. It is becoming increasingly evident that the problem cannot be investigated as one of physical chemistry alone, but must be approached with the recognition that the metabolic and vital processes participate in a mechanism which is peculiar to living systems; although it is not suggested that if a complete analysis became possible the mechanism would contravene fundamental physico-chemical laws. This is well illustrated by two series of investigations in which potassium bromide (because of its neutral, non-toxic nature and the facility with which it may be absorbed and subsequently estimated (11)) has been extensively used.

The first series (12, 14, 15, 16, 17) has been concerned with the inter-nodal cells of the green alga *Nitella clavata* from which vacuole sap may be derived almost uncontaminated by other constituents. It has been emphasized that the cells absorb bromide until high internal concentrations are attained, because they can utilize energy derived from their own metabolism. Since the cells of *Nitella* do not store extensive carbohydrate reserves it was found necessary to maintain this supply of energy by exposure to light during the absorption period (14). Results were obtained which showed a close correlation between the amount of bromide absorbed and the duration and intensity of illumination (17), and there is also some suggestion (12) that oxygen supply may be of importance.



The second series of investigations (29, 30, 31, 32, 33) has utilized cells rich in carbohydrate, namely the storage parenchyma of the potato tuber. With this tissue high internal concentrations<sup>1</sup> are more rapidly attained from dilute solution than in the case of *Nitella*. Again it has been possible to produce a considerable amount of experimental evidence which suggests that the absorption process in potato is much more intimately connected with vital metabolic processes than the older work with storage tissue indicates, and that it is, in fact, conditioned by those variables which also determine the rate of aerobic respiration. In potato the respiration rate may attain unexpectedly high values in the cells at the tissue surface in contact with a sufficient amount of dissolved oxygen (31, 33), and may be maintained over protracted periods (32, 33) at the expense of the aforementioned carbohydrate reserve. Simultaneously other indications of increased metabolic activity accompanied by extensive salt absorption and other processes demanding energy appear in the surface cells. Since the internal carbohydrate supply is adequate, the factor limiting the energy supply to these cells, and with it both respiration and salt absorption, is usually the oxygen concentration in the environment.

The exchange of chloride for bromide is a prominent feature of the absorption of bromide by *Nitella* cells, though the whole amount is not accounted for in this way (17). A similar exchange has been observed in the case of *Valonia* (19). Bromide also penetrates red blood corpuscles readily (39), and an exchange of chloride for bromide is the principal feature of the distribution of bromide between serum and red blood corpuscles (38). Direct exchange of chloride for bromide is, however, a negligible factor in the absorption of bromide by potato tissue (29), therefore it is a matter of some interest to ascertain the behaviour of other storage tissues in this respect.

Should it subsequently appear that the ability to absorb potassium bromide is not a fairly general property of plant cells then the results obtained by its use would be deprived of much of their value, especially for the purpose of the construction of a general theory. Bromide is, of course, a common constituent of marine organisms,<sup>2</sup> though it is, perhaps fortunately, absent (for all practical purposes) from terrestrial plants. This absence is, however, somewhat fortuitous, and is due rather to the rarity of its occurrence in the habitat than to any intrinsic inability of the organism to absorb this salt. Unpublished observations by Hoagland and his collaborators show that the bromide ion may be absorbed readily by the roots of barley (*Hordeum sativum*) plants, and Anne G. Steward, working with one of us (F.C.S.), has found repeatedly that it may be rapidly accumulated by growing marrow (*Cucurbita pepo*) plants without any evident injurious

<sup>1</sup> In this case the internal concentrations refer to expressed saps.

<sup>2</sup> Recall the discovery of bromine by Balard in 1826 in the ashes of seaweeds.

effects.<sup>1</sup> This communication contains the results of an examination of bromide absorption and loss of chloride (if any) by tissue from a variety of plant storage organs representing considerable diversity of morphological character and a wide range of genera.

A complete theory of solute absorption must explain the striking differences of behaviour which often prevail between different plants even when they are exposed to identical solutions. This is a familiar result with higher plants in water cultures (22, 23). The analytical data upon the fluids normally contained in the vacuoles of various marine and fresh-water algae (2, 5, 6, 8, 9, 24) now provide ample evidence that the differences (both of kind and degree) observed are not wholly explicable in terms of environmental factors, but involve also properties of the cells which are characteristic of the species, and are not necessarily identical even for closely related plants.<sup>2</sup> Brooks (6) apparently suggests that the salt content of marine algae may even supplement, if not supersede, purely morphological criteria in their identification. There has been a recent tendency to attribute these highly specific differences between cells to subtle variations in the functional membranes in cells (20, 21, 40). It is true that a much favoured view (18) of the membrane structure which combines elements both of the lipid solubility and ultra-filter theories gives ample scope for highly specific differences. Slight variations in the number, size, and charge of the hypothetical water-filled pores as well as the properties of the otherwise continuous membrane present endless possibilities. Whilst this avenue of approach cannot be neglected entirely, the experiments here reported have been actuated by the idea—which naturally emerged from the preceding investigations already referred to—that differences in the capacity of cells to accumulate salts are more probably associated with fundamental differences of vital function or metabolism than resident exclusively in the properties of a membrane. One might well despair of successful inquiry into the cause of these essentially biological differences solely by analysis of mature cells, the sap-content of which has been attained over relatively long periods and independently of any experimental control. For biological comparisons of this kind plant-storage tissues, which may all be subjected to experiment under completely controlled environmental conditions (30), are particularly suitable; and the more so, inasmuch as it may be inferred with confidence from the results of earlier investigators (35, 36, 37, and papers cited in these works) that various storage tissues will reveal differences in their capacity to absorb salt. To ascribe these properties to manifestations of 'selective permeability' merely

<sup>1</sup> Rosenfels (University of California) has shown in unpublished experiments that bromide may be accumulated by *Elodea*.

<sup>2</sup> The behaviour towards dyes of several species of *Valonia* has been investigated by M. M. Brooks (4).

evades the problem, especially as it becomes increasingly more difficult to relate the so-called permeability properties (as revealed by plasmolytic or tissue conductivity methods) to the absorption process in the normal cell.

The close parallel between the respiratory behaviour of potato tissue and its salt absorption suggested that the differences, if any, in bromide uptake of different tissues might be correlated with similar differences in their total respiration as measured by the carbon dioxide production; although Parker (25) failed to find any direct relationship of this kind for the root systems of different plants.<sup>1</sup> The respiration data in this paper test this point of view.

#### *The storage tissues used.*

Amongst the fleshy plant structures in which organic matter (both soluble and insoluble) is stored in parenchyma cells there is great variety of morphological nature, of storage substance, and of general physiological activity. Such differences are conspicuously displayed by the tissues employed in this investigation. Many, though perhaps not all, of these tissues have previously figured in salt absorption experiments, although the differences between them have not, we believe, been examined by the methods here employed. The investigation concerned solely the more outstanding differences between different storage organs, and was not extended to include a consideration of the smaller ones between varieties, though this might eventually prove profitable.

From the standpoint of the present discussion particular importance attaches to those tissues which remain capable of a further period of growth. The factors which determine the onset of renewed growth, where this is possible, are no doubt complex. The capacity to grow may reside in lateral buds borne upon a structure which is morphologically a swollen stem (e.g. potato, artichoke, and kohlrabi) or again in the renewed activity of an existing cambium or a phellogen regenerated in cells which originated by the division of a similar tissue. All the stem structures cited may, under appropriate conditions, develop renewed activity in both these ways. The dormancy of the resting organ seems to be controlled by the buds, and when these eventually grow they deplete the stored carbohydrate reserves of the parenchyma cells. Detached slices, free from buds, when cut from the dormant organ may still embark upon further growth. The production of a cork cambium at a cut potato surface is familiar (27, 28). Both artichoke and kohlrabi are capable of similar behaviour,<sup>2</sup> though certain minor differences (e.g. rate) are apparent.

The organic reserves of enlarged roots may be depleted during a sub-

<sup>1</sup> Those effects of respiratory carbon dioxide which depend upon solution of otherwise insoluble minerals need not concern us here (10, 22).

<sup>2</sup> Unpublished observations by Miss E. Chamberlain and one of us (F.C.S.).

sequent growth cycle by the development of buds usually borne, not upon the root proper, but at the crown of the storage organ. However, the tissue of the enlarged root, though devoid of buds, may still in many cases actively regenerate at a cut surface exposed to moist air. The details of the process may be somewhat complicated (1, 28, pp. 55-9), and need no full discussion here. Suffice it to say that the somewhat random proliferations producing callus tissue or the more regular divisions of the cork phellogen which may arise are usually most active in tissue nearest the existing cork or vascular cambium, both of which can embark upon renewed activity. In those cases where the storage organ possesses concentric rings of vascular cambium (e.g. beet, &c.) the tissue bordering the outer rings is usually the most active, and this activity tends to spread over the intervening zones of parenchyma cells. With one exception (dahlia tuber) the wound healing of all the root structures used in this investigation had been previously investigated by Bebbington in this Department (see 28). Recent unpublished observations show that slices from dahlia tubers are also capable of healing in moist air. All these tissues do not heal with equal facility. An outstanding case is that of parsnip which heals with extreme difficulty (see 28, p. 58).

The fleshy pericarp of pome fruits (morphologically either enlarged receptacle or the fused bases of floral parts) exhibits different behaviour. Being devoid of buds, or of cambium (except in the vascular strands), and isolated even from the seed, these tissues though composed of living cells, remain incapable of further growth, and their stored carbohydrate and organic acid are not utilized by the plant. Further, they do not even regenerate at a cut surface after they are mature. For example, Chandler (7, p. 647) states: 'The fruit is in a measure at least a living thing. After it reaches maturity it contains apparently no cells that can be caused to divide; and so it can form no new tissue, not even a corky covering for wounds. The changes that take place are primarily those involved in breaking-down of materials; the fruit is destroying itself by its own processes.' Two cases in point are the fleshy tissues of apple and pear.

Certain other structures present slightly different features. Though morphologically modified leaves they are associated with active growing regions (bud or embryo) which may deplete their stored carbohydrate when growth ensues, although no further development of their parenchyma cells is possible. The bulb scales of monocotyledons are composed of tissue of this kind. They are devoid of cambium, and wound cork, relatively rare amongst monocotyledons (26), is at any rate not formed by the isolated tissue of the bulb scales of onion which when isolated from the bud seem to be completely incapable of any further growth. Similar considerations apply to the fleshy cotyledons of leguminous seeds, e.g. pea (*Pisum sativum*), and bean (*Vicia faba*), the living cells of which do not

normally grow even after absorption of water, and remain incapable of further growth if completely severed from the embryo. Certain special cases where cotyledons may regenerate roots need not concern us here (see 28).

These considerations have been set out at some length because it appears that they are associated, in a manner which will subsequently appear, with the behaviour of the tissues in dilute solution, although it is true that the histological changes during wound healing already referred to are characteristic of the behaviour in moist air, and are not completed in a limited period under aerated water.

### *Technique.*

In all cases where massive tissues were used they were cut into circular discs by the procedure described elsewhere (30). The orientation of the circular cylinder of tissue first removed, and consequently of the discs cut from it, was determined rather by convenience than by any morphological consideration. In five cases (mangold, artichoke, dahlia, potato, and kohlrabi) the discs used corresponded to transverse sections of the storage organ—unless otherwise stated the remainder were cut in a tangential plane. It may be assumed that the organs bearing buds were so large that the discs did not actually contain bud tissue. Where a prominent central core of woody tissue (carrot and parsnip) was present or carpels containing seeds (apple and pear) these portions were discarded. The bulb-scale tissue used was derived from the central portion of the larger scales of onion, and the discs, which had the original thickness of the scale, were of the same diameter as those of the other tissues. The epidermis with its impervious cuticle was removed from both surfaces of the onion discs. The cotyledons of bean and pea were derived from seeds swollen for twenty-four hours in distilled water after a preliminary sterilization (in dilute sodium hypochlorite by the method in common use for seeds) and subsequent washing.

The testae were discarded, and the cotyledons separated and used in batches of 100 (i.e. 50 seeds) in the case of pea and 50 for bean (25 seeds) after the embryos had been completely removed. Only very minute and almost negligible growth<sup>1</sup> of unremoved portions of embryo occurred in a few of the cotyledons of pea, and external growth in bean seemed insufficient to warrant removal of any tissue. Wherever possible (all cases except those of the cotyledons) the final salt analyses were made upon the expressed sap, and in the exceptions stated the whole tissue was examined after the usual washing procedure in presence of alkali.<sup>2</sup> In the latter cases the bromide concentrations were calculated as mgm. equivs. per litre of total water in the tissue.

Forty discs of tissue approximately 0.16 cm. thick were placed in two

<sup>1</sup> Removed prior to analysis.

<sup>2</sup> For methods of analysis see (30). NaOH used instead of Na<sub>2</sub>O<sub>2</sub>.

litres of dilute potassium bromide (0.00075 M) solution contained in the special vessels designed for this work (30). The methods for the control of environmental factors and for the determination of carbon dioxide have been fully described elsewhere (30).<sup>1</sup> The experiments (at 23.2° C.) were conducted in three series. In the first, which lasted 91 hours, all the tissues (dahlia, parsnip, mangold, kohl-rabi, carrot, red beetroot, artichoke, and turnip) remained healthy, though certain of the solutions developed slight turbidity. In the second, which was of 104 hours' duration, the tissue<sup>2</sup> (apple and pear) became somewhat disorganized (especially the apple). In the third series (cotyledons of pea and bean and the bulb scales of onion) it was decided to terminate the experiment after 65 hours since the solutions became decidedly turbid.

*Experimental results and discussion.*

The experimental results are presented in Tables I and II and also graphically in the diagram. For comparison with Series I a typical result with potato (obtained on a different occasion, but under similar conditions) is included. Since the unknown specific surface of the tissue pieces in Series III was not identical with that of the tissue in Series I the respiration data are omitted, but since these tissues failed completely to accumulate bromide, these results do not greatly affect the subsequent comparison between respiration and bromide absorption.

The total halide and chloride concentrations in the initial and final expressed sap of the tissues of Series I indicate the probable importance of a direct exchange of bromide for chloride in these tissues. It has been shown previously that the bromide absorbed by potato is not exchanged for chloride, and it is not necessary to recapitulate these results (29, 32). It will be evident that in the case of kohl-rabi, turnip, and artichoke the chloride content was maintained at approximately its original value, despite some considerable bromide absorption, equivalent to several times the original chloride content of the tissue in the two former cases. Some slight reduction in concentration may not imply a loss of chloride by the tissues since they invariably gained a little in fresh weight. These figures remain uncorrected for this factor. Beet and mangold exhibited a significant loss of chloride though less than the bromide absorbed. For these samples of carrot and dahlia tissue the loss of chloride was slightly in excess of the bromide absorbed, whilst in the case of the parsnip it was actually many times greater. There is then very considerable doubt, even in those tissues in which a definite decrease of chloride occurred, whether this was directly related to bromide absorption. The dangers inherent in the mere

<sup>1</sup> All the differences between different tissues subsequently referred to, greatly exceed those between parallel samples of similar tissue (33).

<sup>2</sup> Inadvertently used in discs thinner than series I.

TABLE I.

Tissue.	Mean thickness (cm.).	Fresh weight (40 discs).	Final weight (40 discs).	Period.	Hours.	mgm. CO <sub>2</sub> gm. hrs.
Carrot	0.159	54.20	60.27	1	19.00	0.1411
				2	24.58	0.2080
				3	23.00	0.2161
				4	24.33	0.2105
				1 to 4		0.1967
Parsnip	0.163	53.61	65.40	1	20.33	0.2101
				2	24.25	0.2995
				3	23.50	0.3493
				4	24.25	0.2920
				1 to 4		0.2905
Beet	0.159	60.73	72.82	1	19.00	0.1411
				2	24.58	0.1868
				3	23.00	0.1737
				4	24.33	0.1581
				1 to 4		0.1663
Mangold	0.157	56.72	66.92	1	19.58	0.1288
				2	24.33	0.1649
				3	23.33	0.1395
				4	24.25	0.1410
				1 to 4		0.1537
Kohl-rabi	0.179	65.08	76.97	1	19.58	0.1864
				2	24.33	0.2066
				3	23.33	0.2585
				4	24.25	0.2632
				1 to 4		0.2307
Turnip	0.159	53.95	62.39	1	19.08	0.1639
				2	24.41	0.2024
				3	23.00	0.2218
				4	24.41	0.1948
				1 to 4		0.1972
Artichoke	0.158	55.56	56.44	1	19.08	0.1668
				2	24.41	0.0909
				3	23.00	0.0638
				4	24.41	0.0498
				1 to 4		0.0889
Dahlia	0.157	61.03	62.49	1	20.33	0.1126
				2	24.25	0.1350
				3	23.50	0.1427
				4	24.25	0.1323
				1 to 4		0.1313

TABLE I (*continued*).

Tissue.	Mean thickness (cm.).	Fresh weight. (40 discs).	Final weight (40 discs).	Period.	Hours.	mgm. CO <sub>2</sub> grm. hrs.
Potato	0.157	61.20	69.40	1	17.91	0.1517
				2	23.75	0.1384
				3	25.60	0.1355
				4	26.50	0.1266
				1 to 4		0.1335
Apple	0.075	10.60	9.21	1	22.25	0.0986
				2	24.00	0.0686
				3	24.50	0.0545
				4	24.00	0.0471
				1 to 4		0.0666
Pear	0.075	18.70	12.82	1	22.25	0.0904
				2	24.00	0.0516
				3	24.50	0.0403
				4	24.00	0.0596
				1 to 4		0.0751

TABLE II.

Ext. Conc. KBr. = 0.75 mgm. equivs. per litre.

Series.	Tissue.	Sap concs. mgm. equivs. per litre.			
		Final Br.	Final (Br + Cl).	Final Cl.	Initial Cl.
I.	Carrot	4.63	14.21	9.58	15.92
	Parsnip	1.74	5.22	3.48	13.32
	Beet	14.38	27.90	13.52	15.08
	Mangold	10.40	36.60	26.20	33.24
	Kohl-rabi	12.78	18.61	5.83	6.73
	Turnip	22.73	25.90	3.17	4.83
	Artichoke	20.20	47.51	27.31	24.79
	Dahlia	2.84	11.10	8.26	11.53
	Potato	11.62			
II.	Apple	negligible			
	Pear	negligible			
III.	Onion	0.34			
	Pea cotyl.	0.05			
	Bean cotyl.	0.38 <sup>1</sup>			

comparison of initial and final saps, which cannot segregate those effects which rapidly follow the immersion of the tissue in water from those directly associated with the slow and progressive absorption of salts, have previously been emphasized for the case of potato (29). No doubt these complications will apply in somewhat unknown degree to the tissues in

<sup>1</sup> It is possible that even this amount was localized in unremoved portions of embryo whose growth during the experiment was not conspicuous enough to suggest further dissection prior to analysis as in pea.



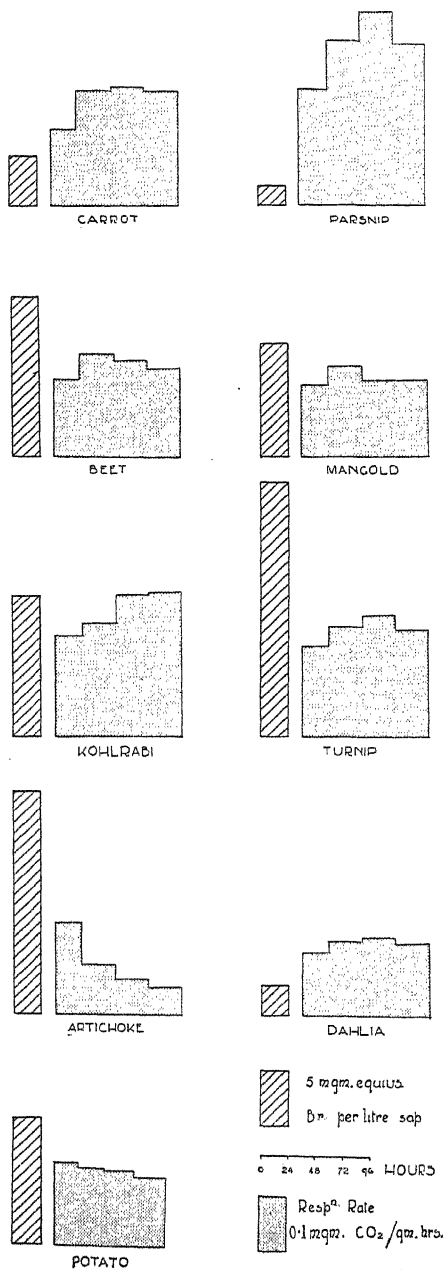
question. There is little doubt (as we shall subsequently stress) that the bromide ion was absorbed by a relatively small proportion of the cells of the discs used. Potato cells which, for other reasons, are not sufficiently active to absorb bromide may, though still living, lose chloride extensively (32). The possibility remains that where an extensive loss of chloride occurred it proceeded mainly from cells not actually absorbing bromide. Apparently then a direct exchange of bromide for chloride, such as that shown by Nitella, is not a prominent feature of the absorption of this ion by storage tissues. Doubtless these tissues (and especially those which sustained but little loss of their original solutes), like potato tissue, absorb bromide associated with the cation potassium. It has been possible to verify this fact in the case of artichoke (34).<sup>1</sup>

A cursory examination of the data might suggest that there is little evidence of any fundamental property of the storage tissues which determines their bromide absorption. It is quite certain that where comparisons between the bromide absorption of *different* tissues are concerned the total carbon dioxide production has not the significance which previous work (31, 32, 33)—and many as yet unpublished results—show that it has when the salt absorption of the *same* tissue under various experimental conditions is in question. The diagram shows that high total respiration and high salt absorption are not invariably associated, in fact, so many combinations of high respiration and low salt absorption, and vice versa, can only preclude any simple explanation along these lines. The tissues also exhibit different behaviour in the drift of respiration with time. Artichoke shows a progressively<sup>2</sup> and rapidly declining respiration rate whilst kohlrabi exhibits a steadily increasing respiration. Between these two extremes other tissues occupy intermediate positions. The relation, if any, between the behaviour of the respiration in time and salt absorption has not as yet been examined in detail except in the most striking case, namely, that of artichoke (see Diagram). One property is common to all these tissues. Without exception they respire over the interval studied at a much higher rate than the original storage organ—another example of the so-called wound respiration. In the case of potato it has been pointed out that this increased respiration is largely conditioned by oxygen supply and is peculiarly localized in a few surface cells. One might confidently anticipate that similar conclusions would also apply to the other tissues of Series I. In the cases yet examined (artichoke, and carrot) this appears to be true. Despite their reduced thickness, which in the light of other work with the tissues of Series I might have been expected to cause much higher respiration rates, the tissues of

<sup>1</sup> The question whether salts with less readily absorbed cations (Ca), or previous treatment with KCl may induce chloride-bromide exchange by storage tissue is receiving further attention.

<sup>2</sup> A much shorter washing and first period would, no doubt, have revealed a brief period during which the respiration was rapidly rising.

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VARIOUS STORAGE ORGANS



Series II exhibited lower and falling respiration rates not greatly in excess of the probable figure for the uncut fruit at this temperature.

In the face of such striking differences in salt absorption and respiration under identical environmental conditions as those shown by artichoke and dahlia it is difficult to avoid designating them 'specific' or 'selective'. Without doubt, however, the *degree* of bromide accumulation must be in part determined by the previous history, variety, &c., of the particular batch of tissue utilized—a matter necessarily somewhat obscure in these experiments. It is not proposed to seek here an explanation for the relatively small differences such as those which obtain between mangold and kohlrabi in Table III, but rather to inquire whether there is any property common to all those tissues which accumulate the bromide ion (without especial regard to the degree of such accumulation); a property in fact which might bestow the *capacity to accumulate* this salt.

It is evident that the storage organs may be divided into two main groups—those which will absorb and accumulate bromide and those which fail to do so, or merely absorb a little bromide but fail even to reach equality of concentration with the external solution. It must be more than a coincidence that all of the nine cases available where the storage tissue accumulates bromide are drawn from storage organs which, as mentioned on p. 398 are still capable of further growth and development by virtue of a cambium or the regeneration which normally occurs at a cut surface exposed to moist air. Without exception the five reported cases (Series II and III) in which the tissue, though subjected to apparently optimum experimental conditions, did not accumulate the bromide ion are also cases in which the tissue is completely incapable of that recrudescence of vital activity which, in the case of the tissues of Series I, leads eventually to meristematic activity and renewed growth when they are exposed to air and moisture. In two of the cases (Series II) where no bromide absorption was obtained the cells once mature normally play no further part in the life-cycle of the plant. Three other examples (Series III), in which the bromide in the tissue aliquot analysed amounted to little more than that required for a positive determination, involve structures (morphologically modified leaves) which are incapable of further growth, though they may release again their storage substance to the plant, but normally only after the development of the bud or embryo, from which they were isolated in these experiments.

Apparently the capacity to accumulate salt rapidly from dilute solution is essentially a property of cells, no longer strictly 'storage' or 'resting' cells, but actually much more active than the mere absence of external signs of growth would indicate. They are, in fact, undergoing such intense vital activity that, unless the duration of the experiment and other environmental conditions impose other limitations, actual cell divisions would

eventuate. Tissue which contains no cells capable of such behaviour, despite a considerable rate of carbon dioxide production, remains incapable of accumulating salt. In any given mass of tissue the proportion of such physiologically active cells will depend very largely upon the storage organ from which it was originally removed. In the case of potato the cells at the surface of a tissue mass respond very uniformly to oxygen in the manner indicated. Parsnip which in Series I shows minimum tendency to absorb bromide, coupled with a very pronounced loss of other solute (chloride), is also a case in which the tissue is known to heal only with the greatest difficulty, whilst artichoke, which absorbed a very considerable amount of bromide is remarkable for the great depth of dividing cells which may appear at a cut surface in air. Using tissue obtained from organs less homogeneous than potato, individual discs, though of the same size and specific surface, may differ greatly in the activity of the cells they contain; since cells closest to the original cambium often display greatest activity. Further there is a strong presumption<sup>1</sup> that these tissues still resemble potato in that the surface cells being most favourably situated to maintain a high respiration rate are also most active in salt absorption. Both these factors will result in unequal distribution of bromide within a single disc. It may be possible ultimately to correlate these more detailed differences with salt absorption, although at present it will suffice for comparisons to be based upon sap concentrations which refer to large numbers of whole discs.

In short it is no longer possible to assume that cut slices of plant storage tissues provide for physiological work, living material in which the complexities due to rapid growth and metabolism may be eliminated. It appears, especially in the case of salt absorption, that these factors are of paramount importance. Even the most recent interpretation by Briggs (3) of the behaviour of cut slices of storage tissues<sup>2</sup> is based upon the older misconception that the constituent cells have reached a static condition in which further changes typical of growing cells play no part. The writers can see no justification for this view, though they recognize that the often unknown extent to which the capacity of the tissue for renewed growth and metabolism was actually effective in past experiments must have been limited by factors, neither carefully controlled nor accurately specified, imposed by the arbitrary experimental conditions adopted. Whether the increased vital activity which is almost invariably associated with the use of a cut surface may be eliminated completely without simultaneously destroying the capacity of the tissue to accumulate salts is an interesting point. The results, both published (32) and unpublished, obtained by changing the oxygen supply to the tissue suggest that the two properties

<sup>1</sup> All those actually referred to resemble the tissues of Series I in that they contain cells still capable of further growth.

<sup>2</sup> The case of artichoke and carrot has been more fully investigated in this regard.

are inseparable. Except in those cases where the storage organ does not contain cells capable of further independent growth the outstanding differences between growing cells and those of 'storage' tissues are of degree rather than kind.

Another property<sup>1</sup> of the cells which absorb bromide is suggestive. All the tissues which accumulate bromide (Series I) either exhibit protoplasmic streaming normally, even in the resting condition (e.g. artichoke, dahlia, mangold, &c.) or they do so in discs cut and exposed to moist air or aerated solutions (e.g. potato). At least in the varieties of apple<sup>2</sup> and pear<sup>3</sup> yet studied, this does not occur extensively, if at all. Protoplasmic streaming does occur in the cells of the cotyledons of the pea and bean, but only after the plumule and radicle have definitely emerged and extensive translocation is taking place. It did not occur in the cotyledons swollen and isolated from the embryo in these experiments. Protoplasmic streaming can be readily seen in the cells of the inner epidermis of the bulb scales of onion; but only with great difficulty, on account of its irregular occurrence and sluggish rate, in the parenchyma cells of the immersed discs of bud scale obtained from *ungerminated* bulbs. It is difficult to avoid the suggestive parallelism between the occurrence of active protoplasmic streaming, an obvious indication of physiological activity and ability to do mechanical work, and the capacity of the same cells to accumulate salts from dilute solution.

The conclusion seems inevitable that the process of salt accumulation by storage tissue is a property, not merely of living cells, but of very active cells—cells embarking upon a renewed cycle of vital activity which would normally culminate in cell division even though this did not actually occur within the duration or under the conditions of these experiments. In this regard storage tissue resembles the other growing tissues which have been observed to absorb bromide. It seems difficult to suppose that the properties which determine salt absorption are resident merely in the constitution of a membrane, except in so far as the membrane, or membranes, form part of the protoplasmic system in which metabolic reactions are rapidly occurring and hence may be the seat of energy exchanges.

#### SUMMARY.

1. An examination has been made under controlled conditions of the respiration and salt absorption of tissue from a variety of plant storage organs exhibiting a diversity of morphological and physiological characters.
2. No simple relation exists between the total respiration and bromide absorption of the different tissues, although such has consistently appeared

<sup>1</sup> The observations upon which this paragraph is based were made by Miss E. Chamberlain.

<sup>2</sup> Bramley's Seedling.

<sup>3</sup> Except very slowly in certain cells just beneath the cuticle.

where this comparison has referred to the same tissue under a variety of experimental conditions.

3. Of the tissues examined, those which contain many cells still capable of renewed growth, as shown by their ability to regenerate in moist air, possess the *capacity* to accumulate the bromide ion, though in different *degrees*, determined by factors not yet specified (except perhaps in the extreme cases). Other living storage tissues incapable of this behaviour completely fail to accumulate bromide.

It appears that there exists some relationship between the capacity to accumulate bromide and the simultaneous occurrence of active protoplasmic streaming (indicative of physiological activity) in the cells.

4. The tissues which absorb bromide do not directly exchange chloride for bromide. Though many of the tissues decreased in chloride content, the two processes seem to be independent.

5. It is emphasized that vital processes, the importance of which has been inadequately realized in the past, determine the behaviour of plant storage tissues in salt absorption experiments. The mere absence of external signs of growth has induced a too facile assumption that the processes of growth and metabolism may be entirely neglected.

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## Notes on Conifers.

### VIII. The Morphology of *Austrotaxus spicata* Compton.

BY

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With twenty-five Figures in the Text.

BESIDES the original description of this interesting Southern Taxad by its discoverer Compton (6), the only morphological work on the plant has been the previous account of some anatomical features of the ovule by Sahni (24) in comparison with other genera of the Taxaceae. Through the kindness of Professor Sahni and Professor Compton some of the slides prepared by the former were lent to me for further examination. I am also much indebted to Professor Compton for the whole of the material, collected on various dates and in several localities, fixed by him at the time for microscopic work, which he very kindly placed at my disposal. This includes one collection of young ovules at about the time of pollination and several collections shortly after fertilization, which would appear to take place a little more than a year after pollination. Unfortunately no collections of the later series include pre-fertilization stages, so that no early pollen-tube structures have been available. There is also a collection of nearly mature male cones.

It will be convenient to consider four aspects of development in turn: (a) The young ovule about the time of pollination; (b) the female gametophyte and early embryogeny; (c) the vascular structure of the ovule at about the stage of (b); (d) the male cone; concluding with (e) a brief discussion of the facts.

#### (a) *The young ovule.*

So far as has been seen the peculiar position of the *Taxus* ovule, terminal on an extremely short lateral shoot of the short primary fertile branch, as first indicated by Strasburger (27) and more fully studied by Pilger (18) and Dupler (11), is not duplicated in *Austrotaxus*. In the latter the ovule appears to be genuinely terminal on the short primary



fertile branch. The material available, however, was insufficient to decide this point conclusively. It is only in very favourable sections of *Taxus*, at a corresponding stage, that the true relation of primary and secondary axes can be followed clearly.

The ovule itself at or near the time of pollination is extremely like that of *Taxus*. Deep in the nucellus is a rather large group of sporogenous cells (potential spore-mother-cells). In the few sections available only one such cell has been found to develop further, but the prevalence of more than one prothallus in the ovule at a later stage suggests the possibility that exceptionally more than one may divide. Fig. 1 shows the general structure of the ovule at this time and Fig. 2 indicates the details of the sporogenous tissue from the same section, in which a linear tetrad can be seen. Fig. 4 shows a binucleate stage in germination of the megaspore,<sup>1</sup> while a 32-nucleate stage is indicated in Fig. 5. Comparison with corresponding stages of *Taxus*, *Torreya*, and *Cephalotaxus* proves a much closer resemblance to *Taxus* than to the other two genera; especially is this the case in sporogenesis, the group of megaspore-mother-cells closely resembling that found in *Taxus*, while in both *Torreya* (7, 21) and *Cephalotaxus* (14) no trace of tapetum is found.

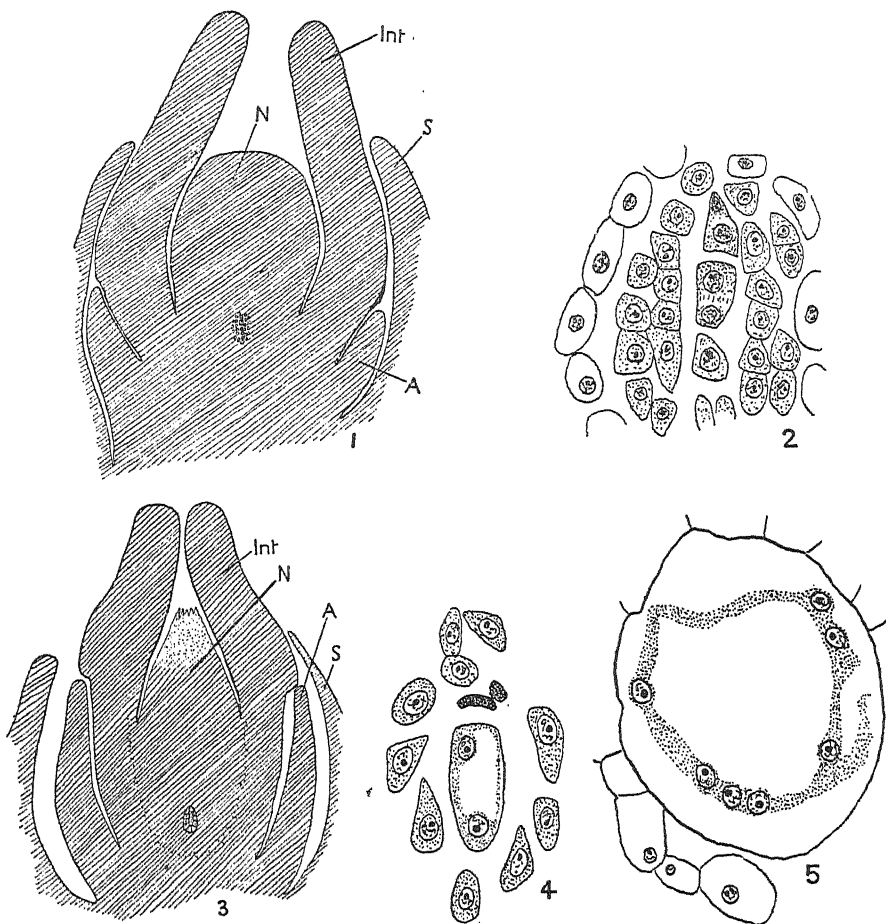
Although the ovule shown in section in Fig. 3 is very probably abortive, no pollen having reached the nucellus, yet it is of considerable interest as demonstrating quite clearly, in comparison with Fig. 1, the region of the ovule which is formed by intercalary growth, namely, that opposite to the free part of the young aril.

(b) *The female gametophyte and embryogeny.*

The early resemblance to *Taxus*, which has just been noted, does not extend to the later stages of development. The gametophyte is much larger than that of *Taxus*, though as in that genus there is a marked tendency for more than one prothallus to develop in an ovule; two such examples are shown in Figs. 9 and 10. The archegonia, too, which are relatively large and conspicuously pointed at the base, resemble those of *Cephalotaxus* much more closely than those of any other genus. Comparison of the archegonia of Figs. 6 and 7 suggests the probability that a ventral canal nucleus is cut off between these stages. Such a nucleus is actually seen in a fair proportion of pro-embryo stages, and it is very probable that the nuclei at the neck end of the archegonia in Figs. 12, 14, and 15 are derived from a ventral canal nucleus. Such a nucleus is undoubtedly present in *Cephalotaxus* (1, 5, 14), but has never been found in *Taxus* and is almost certainly not formed there (9) and was believed to be absent in *Torreya taxifolia* (7), though evidence of its formation was obtained in

<sup>1</sup> The term megaspore is retained, its meaning being perfectly clear, in spite of recent justifiable criticism by Thomson (29, 30).

*T. californica* (22). Besides the larger size of the prothallus and archegonia and the presence of a ventral canal nucleus, there is also, in some cases, an apparent development of the 'tent-pole' structure to which Sahni (25) has

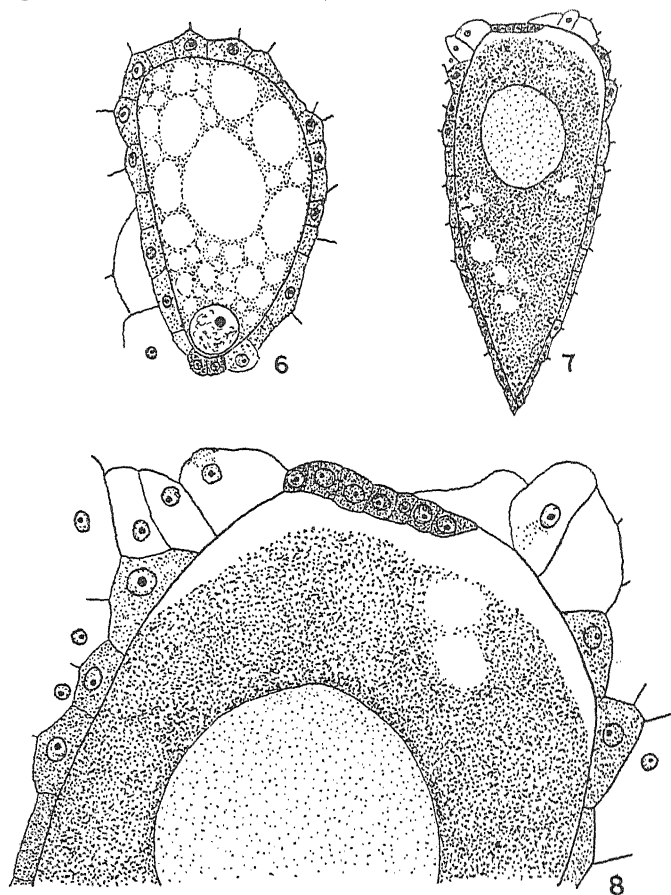


In all A. = Aril, Int. = Integument, N. = Nucellus, S. = Scale.

FIGS. 1-5. 1. Longitudinal section of young ovule, showing position and extent of sporogenous tissue. Aril very short and completely hidden by upper scale leaves. Ready for pollination.  $\times 40$ . Mount Canala, 2,500 ft., 11. 6. 1914. 2. Sporogenous tissue of Fig. 1, showing that the central cell has divided to form a linear tetrad, the rest forming the spongy tissue.  $\times 425$ . 3. Longitudinal section of a somewhat older ovule which is probably abortive, no pollen having reached the nucellus. Note the groove in the integument formed by the pressure of the aril.  $\times 22$ . Locality and date not known. 4. Bi-nucleate prothallus, and a few surrounding cells of the spongy tissue.  $\times 425$ . Mount Canala, 11. 6. 1914. 5. Longitudinal section of a 32-nucleate prothallus.  $\times 335$ . Ignambi, 3,500 ft., 30. 7. 1914.

drawn attention in *Cephalotaxus*. Traces of such a structure may also be seen in *Taxus*, but this is usually only noticeable in quite young stages of the ovule and the pointed apex normally disappears by the time the archegonia are mature. It may be pointed out, however, that in

longitudinal section the 'tent-pole' appears more conspicuous than it actually is if the section passes through an archegonium on either side, since the rather deep depression leading down to the neck of each individual



FIGS. 6-8. 6. Longitudinal section of young archegonium (one of those seen in the small inverted prothallus of Fig. 9), showing the central nucleus immediately below the neck, and the well-marked jacket cells.  $\times 240$ . 7. Mature archegonium. Fertilization has taken place in a neighbouring archegonium but no pollen-tube has reached this one. It is probable that a ventral canal nucleus has been formed and has broken down.  $\times 125$ . Ignambi, 30. 7. 1914. 8. Upper part of Fig. 7, showing character of neck cells, of which sixteen are found in this section and those either side of it.  $\times 425$ .

archegonium appears almost, if not quite, continuous with the pointed apex of the prothallus. It may also be suggested that the comparison with ovules such as that of *Ginkgo*, in which the 'tent-pole' seems to have a definite function in connexion with a free archegonial chamber for swimming sperms, may not be altogether justified.

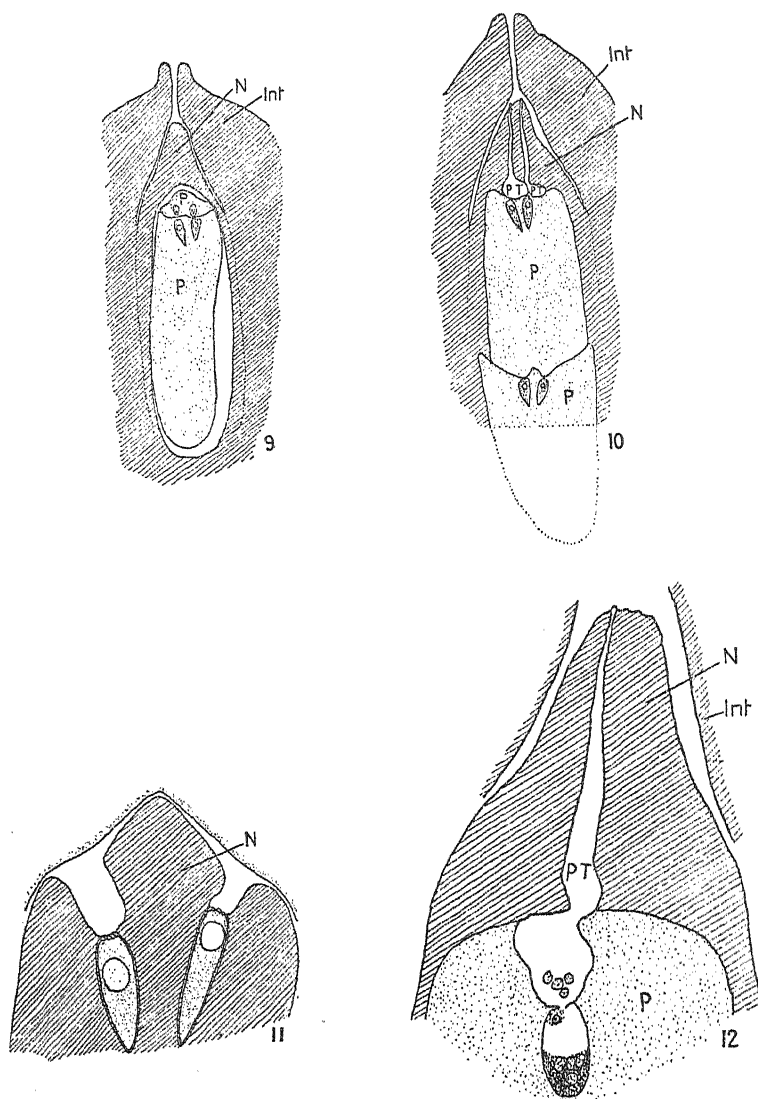
The neck of the mature archegonium (Figs. 7 and 8) is far from conspicuous and cannot always be identified with certainty. In favourable

cases it is seen to be composed of not more than sixteen very small cells, in one plane, quite distinct from the neighbouring cells of the gametophyte. The number of cells in the neck is thus considerably more than in any other genus of the Taxaceae.

The archegonia are always quite separate from each other, and each has a well defined layer of jacket cells (Figs. 6, 7, 8, 14, 15). The number of archegonia usually ranges from three to five and is often four. There may be less in the case of small secondary prothalli, such as that shown in Fig. 9.

The early embryogeny is quite similar to that of *Taxus* (12, 13, 15, 26) and *Cephalotaxus* (1, 3, 5, 14), but the resemblance to *Cephalotaxus* is closer in some respects. No walls are formed until the transition from the 8-nucleate to the 16-nucleate stage, and even then more than half the nuclei remain free until the next division. Figs. 12 and 13 show a 4-nucleate stage, the pro-embryo already only occupying the basal third of the archegonium. In Fig. 12 the adjacent structures are also seen, including the ventral canal nucleus and four nuclei in the pollen-tube. The latter are presumably derived from the division of the vegetative nuclei in the tube, and are not infrequently met with. Fig. 14 shows a similar pro-embryo after the succeeding division. Here also a ventral canal nucleus is seen and the end of the adjacent pollen-tube. In Fig. 15 another division has taken place and the lower seven of the resulting nuclei are now surrounded by walls, while the upper nine lie free in a small mass of cytoplasm. A somewhat similar arrangement of cells and free nuclei is figured by Jaeger (loc. cit.) in *Taxus*. At the upper end of the archegonium seven or eight other nuclei may be seen, the position of which leaves little room to doubt their derivation from a ventral canal nucleus. Four pollen-tube nuclei are also present, as in Fig. 12.

Fig. 16 is of a 32-celled stage, drawn from several serial sections. Careful study of the whole series shows nine rosette cells and nine suspensor cells. Comparison with the preceding figure indicates a strong probability that these result from one more division of the nine nuclei of the upper part of the pro-embryo. The embryo cells below are fourteen in number, and are arranged in four tiers of 5, 5, 3, and 1 cells respectively. From the limited number of preparations available it appears improbable that the arrangement described is quite constant, but on the other hand it is certainly typical and not in any way exceptional. Evidently, therefore, *Austrotaxus* does not closely resemble any other Taxad in these stages of its embryogeny. No evidence has been seen of the casting off of a sterile cap, as described in *Cephalotaxus* by Strasburger (27), Arnoldi (1), Coker (5), Lawson (14), and Buchholz (3). In other respects the later stages of the embryo are very similar to those figured for *Cephalotaxus* by the authors cited, and especially in regard to the formation of a number of rosette



In all Int. = Integument, N. = Nucellus, P. = Prothallus, P.T. = Pollen-tube.

FIGS. 9-12. 9. Longitudinal section of the central part of an ovule with two prothalli, of which the upper is small and inverted.  $\times 10$ . Ignambi, 30. 7. 1914. 10. Section of a similar ovule with two normally oriented prothalli. Part of the lower prothallus is missing in the section and its probable extent is indicated by dotted lines. Two pollen-tubes are seen in the nucellus.  $\times 10$ . Ignambi, 30. 7. 1914. 11. Median section of the upper part of a normal prothallus, showing pointed apex ('tent-pole' structure) and depressions leading to the necks of the archegonia.  $\times 40$ . Ignambi, 30. 7. 1914. 12. A tangential section from the same ovule as Fig. 11, showing nucellar cap, pollen-tube containing four sterile nuclei in the swollen apex, and an archegonium containing a 4-nucleate pro-embryo and another (ventral canal?) nucleus close to the apex.  $\times 40$ .

embryos as described in detail by Buchholz (loc. cit.). Lawson's Fig. 43 might equally well have been drawn from *Austrotaxus*. No evidence of cleavage polyembryony (other than in the rosette embryos) has been seen.

Bi-nucleate and 4-nucleate (rarely 8-nucleate) prothallus cells, especially in proximity to the embryos, are common. In *Taxus* numerous 4-nucleate and a good many 8-nucleate cells are found in a corresponding position.

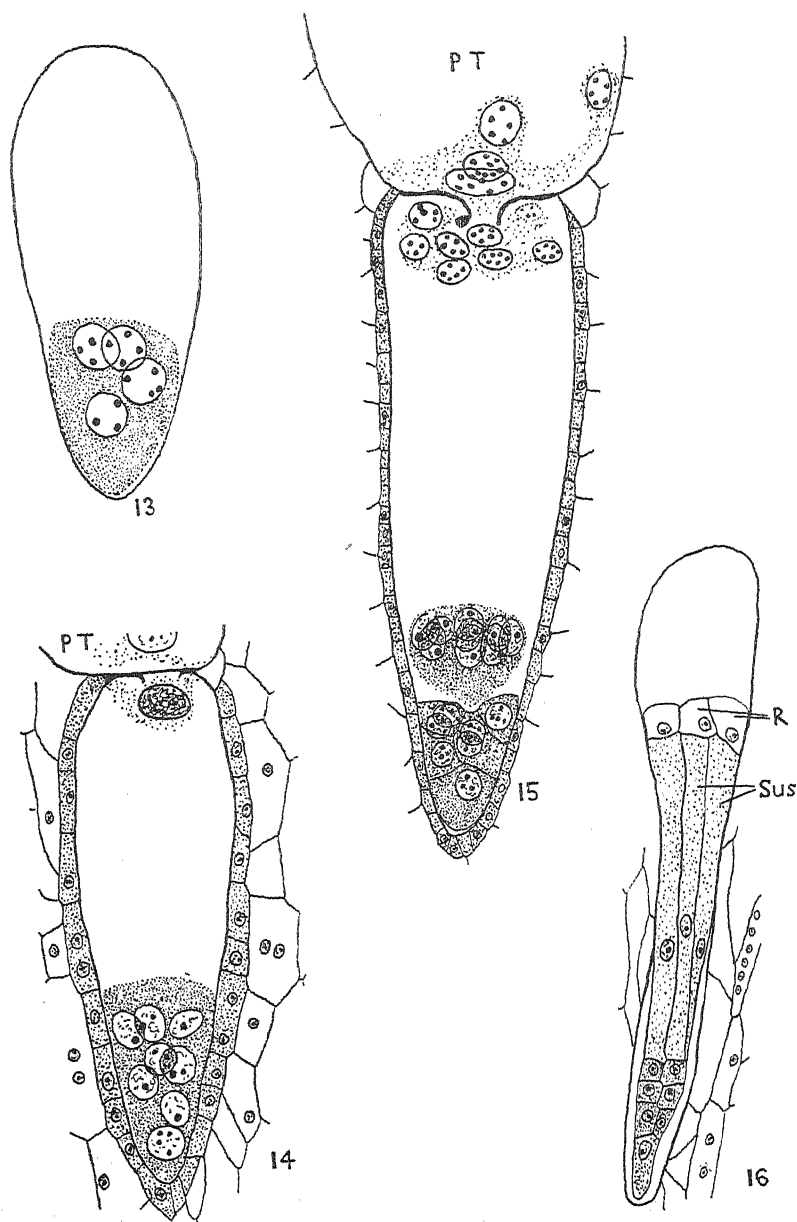
(c) *The vascular structure of the ovule.*

The study of the vascular system has been based largely on the slides prepared by Professor B. Sahni, to which reference has already been made. These include both a longitudinal and a transverse series, almost complete in each case. They were supplemented by another transverse series, cut chiefly to see whether any noticeable variation of structure existed, but this series shows exceedingly close agreement with the earlier set of slides.

Mature seeds not being available, the actual vascular tissue has hardly developed beyond the stage of desmogen strands, though the course and position of these are quite clear and unmistakable. The ovule is slightly flattened in the plane of the main vascular strands, the dimensions of the ovule shown in Fig. 17 being as follows:

Length	. . . . .	6.2 mm.
Breadth in the principal plane	. . . . .	5.7 mm.
Breadth in the plane perpendicular to the preceding	. . . . .	5.0 mm.

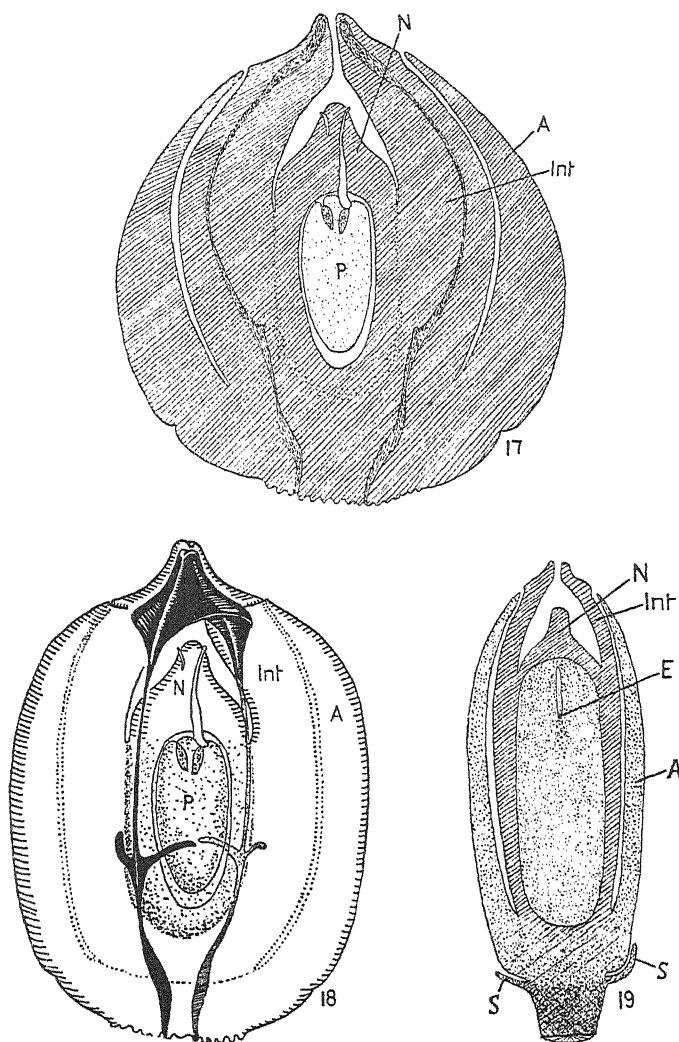
At the extreme base of the ovule is a ring of a few rather diffuse vascular bundles, some of which pass into the uppermost bracts and the remainder appear to end blindly and very probably correspond to the 'basal plate' of *Taxus*. At each end of the longer diameter a small cylindrical vascular strand can be distinguished shortly before the ring of collateral bundles disappears. Each strand bends slightly outwards (Figs. 17 and 18) and thickens somewhat about 0.5–1.0 mm. below the insertion of the aril. On the level of insertion of the aril it becomes very thin and inconspicuous but broadens out again on a level with the base of the prothallus, and immediately above that level it gives off two nearly horizontal branches which pass right and left, each through about one-eighth of the circumference (Fig. 18), while the main bundle bends rather abruptly a little more outwards. The lateral branches lie in the inner region of the integument. Though the integument and nucellus only become separate at a considerably higher level, the junction of the two is very clearly marked by a cylinder of isolated cells with dark-coloured contents (resin cells?) and no vascular tissue of any kind is found in the nucellus. Nor does any vascular strand enter the aril. The two main strands thin out as they pass upwards, but broaden again near the apex, spreading laterally to form a complete perimicropylar girdle of sclerenchymatous cells. To speak of the inner bundle



In all P.T. = Pollen-tube, R. = Rosette, S. = Suspensor.

FIGS. 13-16. 13. An example of a 4-nucleate pro-embryo from the upper prothallus of Fig. 10.  $\times 220$ . 14. 8-nucleate pro-embryo, also showing ventral canal nucleus and end of pollen-tube.  $\times 220$ . Ignambi, 30. 7. 1914. 15. 16-nucleate pro-embryo. The lower seven nuclei have formed walled cells, the upper nine are free in a small mass of cytoplasm. The ventral canal nucleus has proliferated to form, probably, eight nuclei (seven are clearly shown, the eighth doubtfully). Four sterile nuclei are seen in the end of the pollen-tube as in Fig. 12. Drawn from several serial sections.  $\times 220$ . Ignambi, 30. 7. 1914. 16. Longitudinal section of young embryo with suspensor and rosette cells. For explanation see text.  $\times 125$ . Ignambi, 30. 7. 1914.

system as 'nucellar' is clearly incorrect. In the oldest ovules sectioned no lignification of the integument had begun, but it is very probable from



In all A. = Aril, E. = Embryo, Int. = Integument, N. = Nucellus, P. = Prothallus.

FIGS. 17-19. 17. Longitudinal section of whole ovule to show course of vascular strands in the integument.  $\times 10$ . 18. Reconstruction of a similar ovule.  $\times$  about 8. For explanation see text. 19. Median longitudinal section of the largest ovule seen, cut in half and sketched to show relative size and shape of the various parts in comparison with Fig. 17. The embryo is large enough to be readily seen with a hand lens.  $\times 4$ . Igmambi, 13. 8. 1914.

Compton's (6) description that the woody part is on the outside and that all the vascular tissue lies in the inner (fleshy) part of the integument. If this is so, the resemblance to the vascular system of *Taxus* as described by Sahni (24) is a very close one, as he has pointed out. Whether the



rather elaborate theory propounded by him in explanation of the relation-ship between the vascular systems of the ovules in the different genera of the Taxaceae can be accepted is open to doubt. It is generally believed that the ovule of *Torreya* can be explained in the manner suggested by Oliver (17). According to this view all the basal part of the ovule is an intercalated growth and phylogenetically younger than the extreme tip; the junction of the older and younger portions corresponds to the original chalaza. It is difficult to follow Sahni's further elaboration of this idea, particularly in regard to the relation between the vascular system and the woody part of the integument. He says that parts of the vascular strands 'may be imagined to cut their way through the stone, somewhat like a hot piece of wire stretched tight and pressed against a block of wax, so that the regions through which it has passed have again solidified'. It is very hard to believe that any such process can be held to explain the various relations of vascular tissue and sclerotesta in the Taxaceae, nor does there seem adequate ground for the suggestion that the 'entire stone of *Cephalotaxus* corresponds to the newly intercalated portion of the stone of *Torreya* . . .'. Nevertheless there is evidently a close resemblance between the vascular systems of these two genera, particularly in the fact, apparently overlooked by Sahni, that in each case the main vascular supply is in the aril (*Torreya*) or in the outer fleshy part of the integument (*Cephalotaxus*) which is often regarded as homologous with the aril. Arguments based on the gradual shifting of a vascular system between an aril and an (inner) integument hardly seem profitable, and it may be possible to suggest a different comparison. Sahni has himself shown that a rudimentary vascular supply enters the young aril of *Taxus* in the principal plane. Is it not possible that it is this arillar supply which is alone developed in *Cephalotaxus*, while that belonging to the inner integument is missing? In *Torreya* also the main vascular supply would be of this nature, while the branches passing in through the foramina correspond, not to the 'inner system' of *Austrotaxus* but to the outer system in that genus and to the main system of *Taxus*. If this comparison is a valid one then the inner system of *Austrotaxus* has no counterpart in any other genus of Taxaceae. May it not be, further, that overmuch stress has been laid on the morphological aspect of these vascular supply systems, while they have perhaps developed in various ways to satisfy the physiological needs of a moderately large and isolated orthotropous seed? In most conifers the unripe seeds are wholly enclosed in a woody cone and transpiration is probably negligible, but so soon as the growing seed is isolated the problem of a water supply becomes urgent, not only in the case of an ovule with a swimming sperm, as in *Ginkgo* and (doubtless) in various palaeozoic seeds, but also to a less degree in seeds with a siphonogamic mode of fertilization.

Another significant fact apparently not assessed at its true value by Sahni, though he was certainly aware of it, is that the differentiation of the sclerotesta takes place much later than that of the vascular supply, so that instead of the latter forcing its way through the former, the woody part of the integument is really laid down around or within or across the vascular strands, and is interrupted at the points where these strands are already in existence. This is obviously the way that the foramina, both incurrent and excurrent, have been formed.

The general outline of the tissues in the largest ovules available is shown in Fig. 19. It is more than twice as long but very little wider than that of Fig. 17. Growth has been much greater in the prothallus than in any other tissue, its bulk being thirty to forty times, while the general increase in volume is only about three times, that of the ovule of Fig. 17. This increase probably represents about a fortnight's growth.

(d) *The male cone.*

When this paper was nearly completed Professor Compton suggested that a re-examination of the male cones might be useful, since he was not quite satisfied with this part of the original description. It had also been noted that Pilger's (20) description was slightly different to Compton's (6). Compton states that 'the peltate stamens are similar to those of *Taxus*, but are borne in the axils of the bracts of an extended spicate strobilus'; also '*stamina* peltata, 1-5 in bracteae cujusque axillo, microsporangiis 2-4 intus insipientibus, microsporis haud alatis'; while Pilger has '♂ Blüten in kleinen Ähren in den Achseln von Blättern oder Schuppenblättern am Grunde von jungen Zweigen; . . . Blüten mit 1-5 schildförmigen Stam. mit kurzem Filament; . . .'. It is not clear whether Pilger based his description on an examination of specimens of *Austrotaxus*, or whether he interpreted Compton's description in this way; probably the latter. A careful dissection of well-preserved spirit material, supplemented by longitudinal sections of the strobilus, has shown that Compton's description is essentially accurate, but has emphasized the difficulty, already obvious from that description, of accepting the usual morphological interpretation of the structure bearing the pollen-sacs. This difficulty has previously been ably dealt with by Doyle (8), but his reference to *Austrotaxus* is very brief. He remarks that 'it is tempting to look upon it as the "missing link"', i.e. between a primitive strobilus with axillary sporangiophores and the usual type of male cone found in conifers. The present investigation makes that temptation almost overwhelming, though it cannot be denied that another explanation is possible. The resemblance, both in form and structure, between the sporangiophoric structures in *Taxus* and *Austrotaxus* is so close, except for the smaller number of sporangia, usually three, in the latter, that it is certain they are homologous; compare, for instance,

Figs. 21 and 22 with Dupler's (10) figures of *Taxus*. Clearly the current description of the *Taxus* structure as a 'peltate sporophyll' is a misnomer for either genus. It cannot be a leaf for the same reason that the ovuliferous scale of *Pinus*, &c., cannot be a leaf, namely, that one leaf in the axil of another is morphologically impossible. The word peltate implies an umbrella-shaped structure from the under side of which the sporangia hang down. Actually there is no sterile tissue left for them to hang from.<sup>1</sup> In both genera the sporangia alone form the distal part of the structure. In *Austrotaxus* the sporangia are so closely united into groups of (normally) three that each such group might very well be termed a 'synangium', the stalk of the 'synangium' being apparently devoid of recognizable vascular tissue, but as such tissue is certainly present in *Taxus* it is better to follow Doyle and regard it as a sporangiophore. Let it be realized, however, that the sporangia when mature are terminal, in a fused group of three, on the stalk and not pendulous from a peltate expansion. Thus there is little ground for comparison with *Equisetum*, except that the structures borne on the cone axis are in each case radially symmetrical sporangiophores.

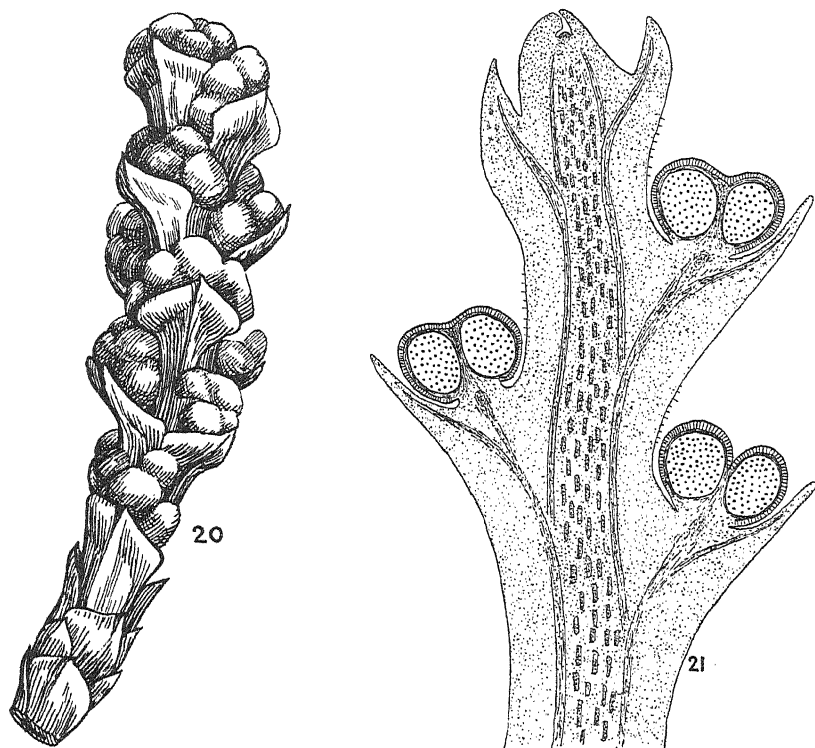
It seems impossible to derive the *Austrotaxus* cone from one of the *Taxus* type, yet the sporangiophores are certainly homologous, and we are therefore forced to the conclusion that *Austrotaxus* is primitive relatively to *Taxus*. Tentatively we may suppose the sporangiophore of Cordaitales to have been near the original type. By fusion of its sporangia and shortening of the stalk we have the condition in *Austrotaxus*, where not infrequently a single sporangiophore is axillary to the bract. Proliferation increased the number of sporangiophores (and later also the number of sporangia in each), and considerations of space made it necessary for sterile tissue to grow up separating the individual sporangiophores, resulting in the *Taxus* male cone. The radial symmetry of the sporangiophore was lost in *Torreya* when some of the potential sporangia developed as resin cavities, and the sterile part of the sporangiophore then became more and more leaf-like, and by this means finally separated the fused sporangia again, as seen in the modern Cupressaceae. The modern view of the leaf as a modified sporangiophore thus derives considerable support from a study of the conifers. It seems probable that the Araucarian male cone does not fit into this sequence, but was itself derived much more directly from some Cordaitalean type.

Reference may now be made to the figures. Fig. 20 is a drawing of the strobilus on a larger scale than that given in Compton's paper. Fig. 21 is slightly diagrammatic, having been drawn from several sections, but gives an accurate idea of the general structure in median longitudinal section. Fig. 22 shows the details of part of one of the sections from which Fig. 21

<sup>1</sup> This refers, obviously, to the *mature* structure. The peltate character of the *very young* sporangiophore is indisputable.

was constructed, and the structure of the pollen grain is indicated in Fig. 23.

The number of bracts subtending sporangiophores in the strobilus

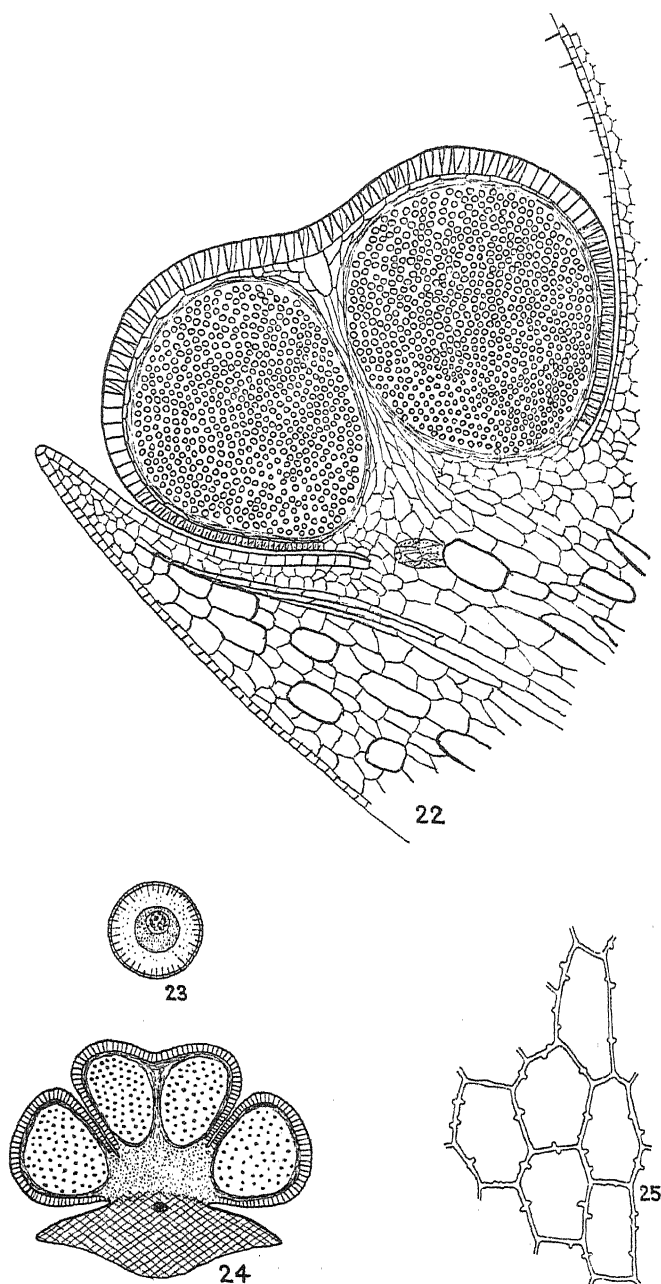


FIGS. 20, 21. 20. Sketch of a complete male cone.  $\times 7$ . 21. Diagrammatic radial longitudinal section of the upper two-thirds of a similar cone.  $\times 12$ .

varies considerably, from three or four to about a dozen. Occasionally a very few sterile bracts are seen at the distal end (apex) of the axis, while several such sterile bracts are always found at the base.

It will be seen that in the two upper fertile bracts of Fig. 21 the section is represented as passing through two of the sporangia of a single sporangiophore, that on the right being cut more medianly than that on the left. In the lowest bract two sporangia belonging to different sporangiophores are drawn, from which it may be seen that the separation between the sporangiophores is not very much greater than that between the sporangia of a single sporangiophore.

Fig. 24 is also diagrammatic, having been drawn from two similar sections, both somewhat broken and incomplete. It has been drawn as representing parts of three sporangiophores, passing through two sporangia of the middle one and one sporangium of each of the laterals.



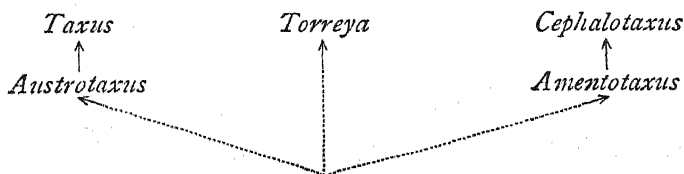
FIGS. 22-5. 22. Part of a similar section.  $\times 48$ . 23. A single pollen grain.  $\times 450$ . 24. Diagrammatic transverse section of a bract (tangential to the strobilus) showing placenta and parts of three sporangiophores.  $\times 17$ . 25. Part of surface section of wall of sporangium, showing characteristic thickening of the radial walls.  $\times 250$ .

Certain further features of interest may be noted in these figures. Figs. 21 and 22: (i) The elongated cells marking the normal position of vascular tissue consist chiefly of parenchyma with very dark contents. Of true vascular tissue there is very little; such as it is, it ends below the sporangiophore. (ii) On the axis just above the insertion of each bract are a number of fine hairs, not cellular, but mere projections from the cuticle. (iii) The sporangium wall includes only epidermis, the outermost 'wall'-layer and a very few flattened and wholly disorganized inner 'wall'-layers. The epidermal cells have radial and slightly spiral thickenings on the walls in the central region of the sporangiophore, and again towards the margin, but about half-way between the two the thickenings appear to be absent (Fig. 22). The nature and extent of the thickenings can be understood by comparison with the surface view of half a dozen epidermal cells shown in Fig. 25. The epidermal cells thus have characters commonly found in a hypodermal endothecium. The pollen grain figured is uninnucleate, but it is not possible to state if it is mature. It is spherical and slightly larger than that of *Taxus* before shedding (29).

#### (c) DISCUSSION.

No very close comparison can be made between *Taxus* and *Austrotaxus* on the one hand and *Cephalotaxus* and *Torreya* on the other as regards the vascular system of the seed; but in some other respects, as already seen, *Austrotaxus* forms a link between the genera *Taxus*, *Cephalotaxus*, and *Amentotaxus*, and a knowledge of its structure confirms the impression that the Taxaceae are a well-defined and distinct family of conifers, the members of which are closely interrelated but show no close relationship with members of any other family. It is now a good many years since Neger (16), followed by Pilger (19), suggested the removal of *Cephalotaxus* and *Amentotaxus* from the Taxaceae as a distinct family. Such a view obtains very little support from any standpoint except that of external morphology, and so far as they go the facts now brought forward are very definitely against it. In fact, excepting the Pinaceae (Abietineae) no family of conifers is more clearly defined than the Taxaceae.

Very tentatively it may be suggested that Taxad phylogeny proceeded from an ancestor combining to some extent the characters of *Austrotaxus*, *Amentotaxus*, and *Torreya*, while *Taxus* and *Cephalotaxus* are more specialized forms derived from *Austrotaxus* and *Amentotaxus* respectively.



A discussion of the more remote relationships of the Taxaceae is outside the scope of this paper.

It will be seen that conceptions of phylogeny based on the general distribution of vascular tissue in the ovule supplemented by a study of the embryogeny and of the microstrobilus do not conform with conclusions based on the distribution of resin cells (2), nor with those of Worsdell (31) founded on the presence of centripetal xylem in the vascular strands of the ovule of *Cephalotaxus*.

The unique character of the male strobilus with its sporangiophores in the axils of bracts has already been stressed.

This work was begun while I was temporarily on the staff of the Botany Department of the University of Cape Town, and I am glad to express my thanks to Professor Adamson for allowing me the privilege of completing it there some time after the termination of my appointment. Lastly, I acknowledge gratefully the assistance of the artist who very kindly prepared Figs. 18 and 20 for me.

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## Notes on Conifers.

### IX. The Ovule and Embryogeny of *Widdringtonia*.

BY

W. T. SAXTON.

MORE than twenty years ago the writer published two rather incomplete accounts of the morphology of *Widdringtonia* (6, 7), with special reference to *W. cupressoides*. In subsequent years opportunities have been taken to continue the study of this species and to obtain material of *W. juniperoides*, *W. Schwarzii*, and *W. Whytei* for comparison. The two latter have been seen only in cultivation, material having been collected from the forestry plantations at Tokai, while collections of *W. juniperoides*, the 'Clanwilliam cedar', were made during a botanical excursion to the Cedarburg in September, 1930, supplemented by further collections very kindly sent to me from that locality during October by Mr. G. J. Oosthuizen, to whom my thanks are due. Two collections were also kindly made for me by Mr. J. B. Cuthbert in May, 1930.

The same difficulties have been experienced as were previously reported in the case of *W. cupressoides*, as although *W. juniperoides* is 'seasonal', in contrast with *W. cupressoides* which is not, yet it varies much, both at different altitudes and from tree to tree, in the stages found on the same date, and in all cases I have failed to obtain any good sections of pro-embryos, in spite of repeatedly getting both slightly younger and slightly older prothalli. Nevertheless, enough has been seen to be worth recording. The only species not examined microscopically is *W. dracomontana* Stapf. Specimens agreeing in every respect with Stapf's species have been seen in the Amatolas, but as they resemble *W. cupressoides* more nearly than do the other three species, it was not thought necessary to investigate the structure.

The ovules at about the pollination stage have been examined in *W. juniperoides*, and the structure has been found to be identical with that of *W. cupressoides*, with a group of potential spore mother-cells. Free nuclear stages of the prothallus have been seen in all species examined,

and these also are indistinguishable from ovules of *W. cupressoides* of similar age. In *W. juniperoides* and *W. Schwarzii* pollen tubes have penetrated some distance into the prothallus. Later stages have been seen only in *W. juniperoides*. Here the lateral archegonia are formed in groups, just as in *W. cupressoides*, but in even larger numbers, sometimes extending through almost the whole length of the prothallus. Over 100 archegonia have been counted in a single longitudinal section of a prothallus. No attempt has been made to count the whole number of archegonia in that prothallus, but it may be estimated at round about 200, far more than in any other conifer investigated. Several prothalli have been seen containing early embryo stages, one- and two- and up to about thirty-celled embryos at the ends of coiled and twisted suspensor cells. As Buchholz (4) has recently pointed out, it would almost certainly be easier to disentangle the embryonic structure from a dissection of living material, but to find suitable stages would involve both very much labour and the destruction of much material in the search for ovules of the right age. Actually only serial sections have been available. Nevertheless it can definitely be stated that the three genera *Actinostrobus*, *Callitris*, and *Widdringtonia* do all agree in their early embryogeny. It was already known that the two former agreed (8, 9), but there was considerable uncertainty about the last. In each case there is cleavage into units, each unit consisting of a single long, coiled, and undivided suspensor cell and a terminal one-celled embryo, the first two divisions in which are normally longitudinal. About eight such units can generally be counted in a prothallus, no doubt arising from the two adjacent pro-embryos, but in one case about fourteen could be made out, probably from an ovule where two pollen-tubes had been functional, thus accounting for four pro-embryos. At a much later stage embryonal tubes form a massive secondary suspensor, as is probably true for all conifers, but no distinction exists between 'prosuspensors' and 'primary suspensors', such as Buchholz has indicated for *Biota* (1), *Sciadopitys* (2), and *Chamaecyparis* (4). In this respect there is a close resemblance to *Cryptomeria* (3), which is said to be 'probably characteristic of Taxodineae', providing yet another point of contact between the Taxodineae and Cupressineae of the Taxonomists, and thus forging another link in the chain of evidence which goes to prove that these two groups should be united in the one family Cupressaceae.

Of a number of nearly mature seeds dissected in May, 1930, two contained two equal embryos side by side. This type of polyembryony is rather infrequent in conifers. A recent account of examples of such polyembryony in some species of *Pinus* is given by Clare and Johnstone (5). The embryo usually has two cotyledons, but occasionally a third smaller one is present.

As this note has been written primarily to clear up some doubtful

points that have recently been called in question by Buchholz (loc. cit.), it has not been thought necessary to add any drawings of the structures concerned.

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# The Relationship Between the Floras of the British Coal Measures and Those of the Stephanian and Permian.

BY

R. CROOKALL, D.Sc.

THE writer has recently published (*a*) accounts of the most characteristic species which compose the four British Coal Measure floras (6, 7), and (*b*) a complete list of the known plant incrustations of the Coal Measures (5). In 1932 he drew up a comparison between the floras of the Lower and Upper Carboniferous, and briefly referred to the older Palaeozoic assemblages of species (4). It is here intended to make a similar comparison with the younger floras which characterize the Stephanian and Permian.

## THE STEPHANIAN FLORA.

The type area of the Stephanian is the Loire (St. Etienne) and the flora has recently been reviewed by Professor P. Bertrand (2). The lowest beds (Faisceau de Rive-de-Gier) are characterized by the frequent occurrence of *Asterotheca lamuriana* (Heer) and of *A. arborescens* (Schlotheim), and by the presence of *Sphenopteris* cf. *cristata* Brongniart, *Zygopteris crosa* Gutbier and *Linopteris duplex* P. Bertrand (a form having affinities with *L. neuropteroides*). The following Westphalian forms persist into the beds: *A. abbreviata* (Brongniart), *Eupecopteris* cf. *dentata* (Brongniart), *Renaultia* aff. *chaerophylloides* (Brongniart), *Sphenophyllum emarginatum* Brongniart, *S. majus* (Bronn) and *Asolanus camptotaenia* Wood, while ribbed Sigillarias of the *S. deutschii*, *tessellata*, and *scutellata* types are frequent.

The overlying Assise de St. Etienne contains many characteristic species, all of which are of more or less common occurrence. The lower beds contain an intermediate flora in which *Asterotheca lamuriana* becomes extinct and *Cordaites lingulatus* Grand'Eury first appears. In the succeeding zone *L. neuropteroides* Goeppert and *Odontopteris macrocephala* P. Bertrand are typical. Above this is the zone of *L. germari* Giebel, while the

topmost beds are characterized by a predominance of *Poacordaites* (i.e. narrow linear Cordaitean leaves with obtuse apices). *O. minor* Brongniart also occurs.

The Assise de St. Etienne is rich in fern-like plants; all being either fairly or very frequent. They include *O. reichi* Gutbier, *O. brardi* Brongniart, *Alethopteris grandini* Brongniart, *Callipteridium pteridium* Schlotheim, *C. gigas* Gutbier, *Zygopteris pinnata* Grand 'Eury, *Diplotmema busqueti* Zeiller, *Neuropteris cordata* Brongniart, *Linopteris brongniarti* Grand 'Eury, *L. germari* Giebel, *Asterotheca cyathea* (Brongniart), *A. lepidorachis* (Brongniart), *A. hemitelioides* (Brongniart), *Pecopteris feminaeformis* Schlotheim and *P. bioti* Brongniart. To these may be added *Cordaitea lingulatus* Grand 'Eury, *Dorycordaites affinis* Grand 'Eury, *Poacordaites linearis* Grand 'Eury, *Sphenophyllum oblongifolium* Germar, *Sigillaria brardi* Brongniart &c. In this division certain Permian forms appear: *Walchias* (*W. piniiformis* Schlotheim, rather frequent), *Taeniopteris* (*T. jejunata* Grand 'Eury), *Pterophyllum* (rare) and *Sphenophyllum thoni* Mahr, while the ribbed *Sigillarias* become extinct. *Cordaitea lingulatus*, a most characteristic species, appears in the lower portion of the Assise de St. Etienne, attains a maximum, and diminishes in frequency towards the top. There is also a number of *Neuropteris* with large pinnules. Of these, *Linopteris neuropteroides* Geoppert and *Odontopteris macrocephala* P. Bertrand are rather frequent in the lower and *Linopteris germari* in the upper portion.

Some few of the above-mentioned species have been found in the British Coal Measures, and especially in the Upper Coal Measures or Radstockian Stage. Arber (1) compared the floras of the British and French Upper Coal Measures, and concluded that there is no palaeobotanical evidence for the occurrence of beds of Stephanian age in Britain. The writer (3) re-examined this question in the light of subsequent records and arrived at the same conclusion. Professor P. Bertrand has expressed verbal agreement with this result. In the following table is shown the occurrence in Britain of the species cited above.

In addition, it should be pointed out that there are many species present in the Upper Coal Measures of Britain which are unknown from the Stephanian, though numerous other forms are common to both.

Arber recognized the following nine species as occurring both in the higher part of the Westphalian and in the Stephanian: *Annularia stellata* (Schlotheim), *A. sphenophylloides* (Zenker), *Sphenophyllum emarginatum* Brongniart, *Asterotheca arborescens* (Schlotheim), *A. cyathea* (Schlotheim), *Acitheca polymorpha* (Brongniart), *Ptychocarpus unitus* (Brongniart), *Alethopteris grandini* (Brongniart) and *Cordaitea angulosostriatus* Grand 'Eury. Remarks on the range in Britain of these plants were given by the writer (3, p. 65).

More or less common Stephanian plants. Occurrence in British Coal Measures.

PTERIDOSPERMEAE AND FILICALES.

<i>Alethopteris grandini</i> Brongniart . . .	? absent
<i>Asterotheca cyathea</i> (Brongniart) . . .	fairly common
„ <i>hemitelioides</i> (Brongniart) . . .	very rare
„ <i>lamuriana</i> (Heer) . . .	very rare
„ <i>lepidorachis</i> (Brongniart) . . .	rare
<i>Callipteridium pteridium</i> Schlotheim . . .	very rare
„ <i>gigas</i> Gutbier . . .	? absent
<i>Diplotmema busqueti</i> Zeiller . . .	absent
<i>Linopteris brongniarti</i> Grand 'Eury . . .	absent
„ <i>germari</i> Giebel . . .	absent
„ <i>neuropteroides</i> Goeppert . . .	absent
<i>Neuropteris cordata</i> Brongniart . . .	absent
<i>Odontopteris brardi</i> Brongniart . . .	absent
„ <i>macrocephala</i> P. Bertrand . . .	absent
„ <i>reichi</i> Gutbier . . .	absent
<i>Pecopteris bioti</i> Brongniart . . .	very rare
„ <i>feminaeformis</i> Brongniart . . .	absent
<i>Taeniopteris jejuna</i> Grand 'Eury . . .	absent
<i>Zygopteris pinnata</i> Grand 'Eury . . .	absent

LYCOPODIALES.

<i>Sigillaria brardi</i> Brongniart . . .	rare
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CORDAITALES.

<i>Cordaites lingulatus</i> Grand 'Eury . . .	absent
<i>Poacordaites affinis</i> Grand 'Eury . . .	absent
„ <i>linearis</i> Grand 'Eury . . .	absent

SPHENOPHYLLALES.

<i>Sphenophyllum oblongifolium</i> Germar . . .	absent
„ <i>thoni</i> Mahr . . .	absent

CYCADOPHYTA.

<i>Pterophyllum</i> spp. . . . .	very rare
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CONIFERALES.

<i>Walchia piniformis</i> Schlotheim . . .	very rare
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Arber further cited the following sixteen species which were common in, and regarded as characteristic of, the Stephanian, and which were (then) unknown from the British Coal Measures: *Calamites gigas* (Brongniart), *Sphenopteris cristata* Brongniart, *Pecopteris bioti* Brongniart, *Asterotheca lepidorachis* (Brongniart), *A. hemitelioides* (Brongniart), *Pecopteris feminaeformis* (Schlotheim), *Callipteridium pteridium* (Schlotheim), *C. gigas* (Schlotheim), *Neuropteris cordata* Brongniart, *Taeniopteris jejuna* Grand 'Eury, *T. multinervis* Weiss, *Zamites carbonarius* Zeiller & Renault, *Plagiosamites planchardi* (Renault), *Pterophyllum fayoli* Renault, *P. grand'euryi* S. & M., and *P. cambrayi* Renault. As the writer (18) has pointed out, some of these species have since been found to occur, very rarely, in Britain (*Pecopteris bioti*, *A. lepidorachis*, *A. hemitelioides*, and *Callipteridium*

*pteridium*), while a few (e.g. *Taeniopteris jejunata*) are more typical of the Permian than of the Stephanian.

#### PERMIAN FLORAS.

The Permian rocks of Great Britain, chiefly represented in the north-east of England by the Magnesian Limestone which stretches from Northumberland to Nottinghamshire, were mainly deposited on the bed of a sea which was subject to desiccation. West of the Pennines, the Magnesian Limestone is much thinner, and the Permian is largely made up of the Penrith Sandstone and the breccias known as the Brockram. These rocks are also the result of arid conditions but, unlike the limestone, which is largely a chemical precipitate, are of terrestrial origin, consisting of land waste. The inclusion of plants in rocks of such origin would be largely accidental, and their occurrence as fossils therefore sporadic. Fossil plants are usually confined to shaly bands, as, for instance, the Marl Slate of the eastern area and the Hilton Plant Beds of the Appleby Region in the West (10). From these beds a few examples of *Walchia*, *Ulmannia*, *Odontopteris*, and *Alethopteris* have been recorded, but some of the records require confirmation. More varied floras occur in the Permian rocks of the Continent.

The Lower Permian plants of the northern hemisphere (somewhat distinct from the Upper Permian flora) include Carboniferous genera. Pteridosperms and ferns and *Calamites* are common, though *Sigillaria* and *Lepidodendron* are rare. *Cordaites* persists, *Walchia* reaches its maximum development, and members of the Ginkgoales (*Saportaea* and *Baiera*) appear. In the Upper Permian of the northern hemisphere the flora contains no examples of *Sigillaria*, *Lepidodendron* or *Calamites*. It has a Mesozoic character. The Shansi (Central China) flora (9) includes forms which are transitional between the late Palaeozoic and the early Mesozoic types.

Typical Permian species on the Continent (8) include *Callipteris conferta* Brongniart, *C. naumanni* (Guthrie), *C. lyratifolia* Goepfert, *Walchia piniformis* Grand'Eury, *Taeniopteris jejunata* Grand'Eury, *T. multinervis* Weiss, *Odontopteris osmundaeformis* (Schlotheim), *Pterophyllum blechnoides* Sandb., *Calamites gigas* Brongniart, *Sphenophyllum thoni* Mahr, *Dicranophyllum* spp. and *Ulmannia* sp. None of these is known from the Coal Measures of Britain, with the single exception of *W. piniformis*, which occurs very rarely.

These forms are accompanied by (a) a few Triassic types (e.g. *Voltzia*) and (b) species which have persisted from the Coal Measures and Stephanian (*Alethopteris grandini*, *Asterotheca arborescens*, *A. candolleana*, *A. hemitelioides*, *A. miltoni*, *Acitheca polymorpha*, *Linopteris germari*, *Callipteridium gigas*, *Pecopteris feminaeformis*, *Ptychocarpus unitus*, *Sphenophyllum ob-*



*longifolium*, *Calamites suckowi*, *C. congeniens*, *Annularia stellata*, *A. sphenophylloides*, *Asterotheca equisetiformis*, *A. longifolius*, *Sigillaria brardi* etc.)

As a number of species range through more than one division, the floras must be taken as a whole. A brief comparison of some of the diagnostic species is given below.

Coal Measures (of Britain).	Stephanian (of continent).	Permian (of continent).
PTERIDOSPERMEAE AND FILICALES.	<i>Acithea polymorpha</i> (Brongt.)	
<i>Alethopteris serli</i> (Brongt.)	<i>Alethopteris grandini</i> (Brongt.)	
<i>A. lonchitica</i> (Schl.)		
<i>Asterotheca miltoni</i> (Artis)	<i>Asterotheca arborescens</i> (Schl.)	
	<i>A. candolleana</i> (Brongt.)	
	<i>A. cyathea</i> (Brongt.)	
	<i>A. hemitelioides</i> (Brongt.)	
	<i>A. lamuriana</i> (Heer)	
	<i>A. lepidorachis</i> (Brongt.)	
	<i>Callipteridium gigas</i> (Gutb.)	
	<i>C. pteridium</i> (Schl.)	
	<i>Dicksonites pluckeneti</i> (Schl.)	
<i>Linopteris münsteri</i> (Eichw.)	<i>Linopteris brongniarti</i> G. 'E.	
<i>L. obliqua</i> (Bunb.)	<i>L. germari</i> Giebel	
	<i>L. neuropteroides</i> Goepp	
<i>Neuropteris ovata</i> Hof.	<i>Neuropteris cordata</i> (Brongt.)	
<i>N. scheuchzeri</i> Hof.		
<i>N. rarineruis</i> Bunb.		
<i>N. tenuifolia</i> Schl.		
<i>N. gigantea</i> Brongt.		
<i>Odontopteris lindleyana</i> (L. & H.)	<i>Odontopteris brardi</i> Brongt.	
	<i>O. reichi</i> Gutb.	
	<i>Pecopteris bioti</i> Brongt.	
	<i>P. feminaeformis</i> Brongt.	
	<i>Ptychocarpus unitus</i> (Brongt.)	
<i>Sphenopteris dilatata</i> L. & H.	<i>Sphenopteris cristata</i> Brongt.	
<i>S. nummularia</i> Gutb.		
<i>S. neuropteroides</i> (Boul.)		
<i>S. striata</i> Gothan		
<i>S. furcata</i> (Brongt.)		
		<i>Callipteris conferta</i> Brongt.
		<i>Taeniopteris jejuna</i> G. 'E.
		<i>T. multinervis</i> G. 'E.
LYCOPODIALES.		
Ribbed Sigillarias, e.g.	Unribbed Sigillarias, e.g.	
<i>Sigillaria deuschi</i> Brongt.	<i>Sigillaria brardi</i> Brongt.	
<i>S. scutellata</i> Brongt.		
<i>S. tessellata</i> Brongt.		
<i>S. mammillaris</i> Brongt.		
<i>S. elegans</i> (Sternb.)		
<i>S. laevigata</i> Brongt.		
<i>S. ovata</i> Sauv.		

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Coal Measures (of Britain).	Stephanian (of continent).	Permian (of continent).
<i>Lepidodendron lycopodioides</i> Sternb.		
<i>L. obovatum</i> Sternb.		
<i>L. ophiurus</i> (Brongt.)		
<i>L. wortheni</i> Lesqx.		
SPHENOPHYLLALES.		
<i>Sphenophyllum emarginatum</i> Brongt.	<i>Sphenophyllum oblongifolium</i> Germ.	<i>Sphenophyllum thoni</i> Mahr
<i>S. cuneifolium</i> (Sternb.)		
<i>S. majus</i> (Bronn)		
<i>S. myriophyllum</i> Crép.		
<i>S. trichomatosum</i> Stur.		
EQUISETALES.		
<i>Annularia sphenophylloides</i> (Zenk.)	<i>Annularia stellata</i> (Schl.)	
<i>A. stellata</i> (Schl.)		
<i>A. galioides</i> L. & H.		
<i>A. radiata</i> Brongt.		
<i>Calamites undulatus</i> Sternb.		<i>Calamites gigas</i> Brongt.
<i>C. sachsei</i> Stur.		(Lower Permian)
<i>C. carinatus</i> Sternb.		
CORDAITALES.		
<i>Cordaites principalis</i> (Germ.)	<i>Cordaites lingulatus</i> G. 'E.	
<i>C. borassifolius</i> (Sternb.)	<i>Poacordaites affinis</i> G. 'E.	
	<i>P. linearis</i> G. 'E.	
CYCADOPHYTA.		
		<i>Pterophyllum</i>
CONIFERALES.		
		<i>Walchia piniiformis</i> G. 'E.
		<i>Dicranophyllum</i> <i>Ulmannia</i>
GINKGOALES.		
		<i>Baiera</i> <i>Saportaea</i>

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# Penetration and Development of the Fungus, *Beauveria Bassiana*, in the Tissues of the Corn Borer.<sup>1</sup>

BY

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With Plate VIII and two Figures in the Text.

## INTRODUCTION.

PRELIMINARY cultural and inoculation experiments with *Beauveria Bassiana* (Bals.) Vuill., a fungus attacking the European corn borer, have been described in a previous paper (7). Later, Bartlett and Lefebvre (1) published the results of field experiments in which this fungus was used as a means for controlling this destructive insect. From these and other experiments, it was obvious that the fungus readily attacked and killed the corn-borer larvae, but the actual relationship between the fungus and its host was not understood, though the macroscopic characteristics of the diseased insects were consistently striking. Therefore, experiments were undertaken to learn by what means *B. Bassiana* gains entrance to its host and to determine its subsequent development in relation to the various tissues of the corn-borer larvae.

## LIFE-HISTORY OF THE CORN BORER.

According to observations by Caffrey and Worthley (3) made since the discovery of the European corn borer in the United States in 1917, there are usually two complete generations developed each year in New England. The insect passes the winter as a full-grown larva or borer within the tunnel made in its host or shelter plant during the previous summer and fall, and the presence of such borers may be detected readily by the small holes on the surface of infested plants, from which extrude masses of frass and casings of the borers.

About the middle of May, the borer cuts a small circular opening from its tunnel to the surface of the plant in order to provide an exit for the future moth. The insect then closes this hole with a thin partition of silk and after retreating into its tunnel, spins a thin cocoon, inside of which it changes into the pupa or resting stage. After remaining in this condition

<sup>1</sup> Contribution from the Laboratories of Cryptogamic Botany, Harvard University, no. 118.

for several weeks, or until about the first week in June, the skin of the pupa splits and the fully developed adult moth emerges.

Soon after emergence, the moths mate and begin to deposit eggs in flat, irregularly shaped masses usually composed of from 15 to 20 eggs, although occasionally much larger numbers have been found.

The eggs hatch in from 4 to 12 days with an average of about 7 days, the duration of the egg stage varying with the climatic conditions. The young larva feeds for a few days upon the surface of the leaf near its place of hatching, but soon enters the plant through the tassel and through the leaf axils and completes most of its development in the stalk. It may, however, migrate to other plants by crawling or by swinging from a suspended thread which it has previously spun. By the third week of July or about 38 days after hatching from the egg, the borer becomes full grown and changes to the pupa, or resting stage, usually inside its tunnel in the host plant. During the last part of July or in early August, the moths emerge from these pupae and deposit their eggs by the method already described above.

The eggs of this generation hatch in about 7 days, and the resulting borers attack the plant in a manner similar to that described for the first generation. At this time, however, many of the newly-hatched borers make their way directly into the ears of the field corn and late sweet corn, and feed within the partly developed ear, thus causing a great deal of injury. The borers feed until their activities are stopped by cold weather in November or early December and remain throughout the winter in a hibernating condition within their tunnels in corn-stalks, corn-cobs, weeds, crop remnants, or other hosts.

The time at which moths lay their eggs varies greatly, depending for the most part on climatic conditions. Hence, if insecticides or methods of biological control such as parasitic fungi are to be employed effectively, it is of utmost importance to pay close attention to the development of these eggs in order to learn when hatching begins, and to ascertain the time at which the peak of hatching has been reached. When the egg-hatching period is at its peak, many of the young larvae are already crawling about and feeding on the leaves of the corn plants. It has been found that this is the most opportune time for the application of the fungus.

In view of the fact that the corn borer spends most of its life within the humid tunnels it makes in the corn-stalk, it seems that the conditions there would be very favourable for the growth of the fungus. Yet the very fact that the insect spends so much of its time within the host plant and that the fungus necessarily must be applied to the surface, makes it advisable to make a study of the life-history of the fungus in relation to the corn borer, in order to understand fully when and how *B. Bassiana* is able to infect and destroy its host.

*Course of Infection.*

*Inoculation.*

The corn-borer larvae studied to determine the manner by which the fungus entered the host were usually dusted with the spores of *Beauveria* or were inoculated by being allowed to crawl over a Petri dish culture of the fungus. Also, in several instances the larvae were inoculated by injecting a spore suspension of the fungus through the anal aperture with a very fine pipette. When the last method was employed, the insects were dipped in 70 per cent. alcohol immediately after the spores had been injected and then passed through several changes of sterile distilled water in order to ensure the removal of any spores which might have adhered to their surface.

Measured amounts of inoculum were not used in each case since it was found that certain uncontrollable factors were involved which made this impracticable. When the inoculum is applied externally, it is difficult to determine exactly the number of spores that are involved in accomplishing infection, since the spores placed on the larvae in the moist chamber germinate and within 36 hours produce myriads of secondary conidia that might also be involved in infection. The study of infection phenomena also was made more difficult by the saprophytic development of the fungus on moist filter paper in the moist chamber and a resulting heavy production of secondary conidia on that material. Moreover, in most cases after inoculation, probably because of the handling they had received, the larvae were very active, so that much of the inoculum was either rubbed off or dislodged from the surface to which it was applied hence, adding to the uncertainty of the ultimate fate of the original inoculum.

*External appearance of the diseased larvae.*

The infected larvae first show evidence of their diseased condition by becoming rather sluggish, failing to respond readily to such external stimuli as light or irritation by needle pricks; and also, in most instances, by turning a pink colour which is a characteristic symptom of the disease. In some cases, however, this pink colour does not appear even though the white fungous hyphae may be seen growing on the skin of the larvae. Later, following thorough penetration of internal tissues by the fungus, the larva generally turns distinctly pink and remains soft and pliable until the mycelium has ramified and grown throughout the various parts of the body. After this, the body becomes rigid—invariably showing the characteristic pink discoloration—and finally in this mummified condition the larva becomes very brittle, so that it can be readily broken and the completely whitened and chalky contents may easily be crumbled into powder. Upon microscopic examination this powdery mass may be seen to be completely

filled with fungous mycelium. When such a mass of insect tissue and mycelium disintegrates in the field in the spring, a great quantity of inoculum is undoubtedly furnished to spread the disease among other insects. This mummification of the larvae is in contrast to the effect produced by members of the Entomophthoraceae (8) which usually render their hosts soft and flaccid, although there is an exception in the case of *Massospora* which, as Speare (9) and Goldstein (5) have found, affects its locust host in such a way that the posterior part of the insect dissolves and sloughs off. If the mummified corn-borer larvae are kept in a dry atmosphere, no external signs of the fungus are evident, but soon after exposure to moist air the white mycelium of the fungus becomes apparent over the surface of the larvae, and after one or two days, conidia are produced in abundance, giving the diseased insects a mealy, powdery appearance.

#### *Penetration of the fungus.*

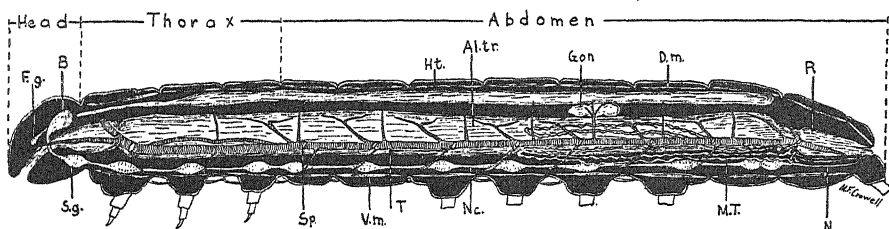
In order to study the actual method by which *Beauveria* penetrates its host, large numbers of healthy larvae collected from various parts of New England were inoculated, and sectioned after the fungus had incubated for varying lengths of time.

In order to avoid the loss of germinating spores, the inoculated corn-borer larvae which were to be sectioned were first anaesthetized with ether to prevent their struggling in the killing fluid. Various killing fluids were used but the most satisfactory proved to be Carnoy's (4) because of its rapid penetration and immediate lethal effect. The larvae, while in the killing fluid, were freed from as much air as possible by the use of an air pump, and in addition, the larger ones were pricked with a needle or sometimes cut in two, in order to ensure more thorough penetration of the killing and fixing fluid. To avoid the difficulty of the larva's becoming hard and brittle, the result first experienced with the standard technique of killing and fixing, the method described by Zirkle (11) in which N-butyl alcohol replaced ethyl alcohol, was used successfully. In some cases when a large number of larvae from a particular lot had to be sectioned, the celloidin method developed by Jeffrey (6) and more recently elaborated by Wetmore (10) was used with equal success. In this way, at least five whole larvae could be sectioned at one time. The sections were cut from 5 to 10 microns in thickness, and were stained with Heidenhain's iron-haematoxylin, eosin being used as a counter stain.

The time required for the fungus to penetrate the body of the host varied with the different larvae examined. This was possibly due to the fact that certain larvae were more active than others, thus interfering to some extent with the vigour of fungal development, or to the fact that some larvae were so placed in the Petri dish that the invading fungus had more favourable opportunities for growth.



The various organs and tissues of a corn-borer larva mentioned in the discussion of fungous penetration are illustrated in the accompanying diagram (Text-fig. 1).



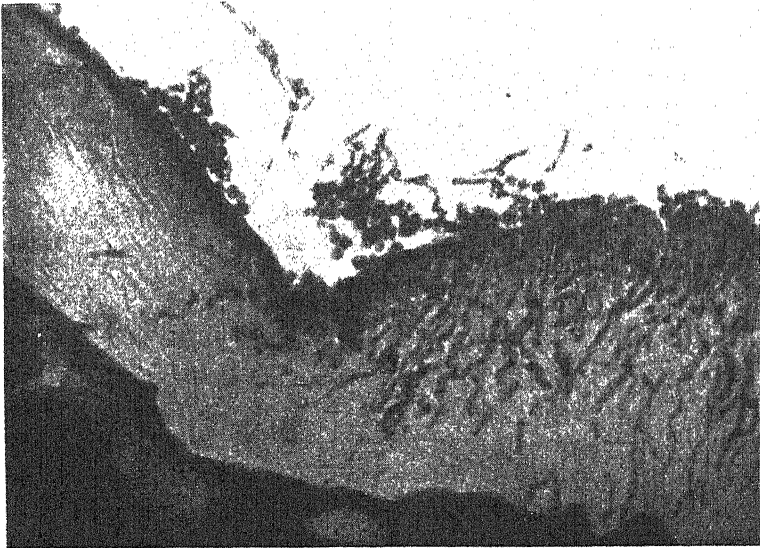
TEXT-FIG. 1.<sup>1</sup> Diagrammatic drawing of a corn-borer larva in sagittal section, showing *f.g.*, front ganglion; *b.*, brain; *s.g.*, subesophageal ganglion; *v.m.*, ventral muscle; *t.*, trachea; *nc.*, nerve cord; *ht.*, heart; *al.tr.*, alimentary tract; *gon.*, gonad; *d.m.*, dorsal muscle; *r.*, rectum; *m.t.*, malpighian tubule; and *n.*, nerve.  $\times 6$ .

The writer finds that the fungus is capable of passing directly through the thick sclerotized body wall of the corn-borer larvae (Pl. VIII, Figs. 2-4) as it seems to produce substances that are capable of dissolving and breaking down the chitinous layer (Text-fig. 2). There also seems to be a mechanical action involved as the layers of the chitin were often somewhat indented in advance of the penetrating hyphae. Soon after penetrating the body wall, the fungus attacks the cells of the fat bodies (Pl. VIII, Fig. 4). The cell walls are pierced, the nuclei soon disintegrate, and the whole mass becomes permeated with mycelium that attains a diameter much greater than that of the hyphae which penetrated the body wall (Pl. VIII, Fig. 8). This tissue of the fat body seems therefore to furnish an excellent medium for the rapid and abundant development of the fungus, probably because of the fats and slightly nutritive nitrogenous material stored therein. However, this pronounced thickening of the mycelium is not limited to the cells of the fat bodies, but holds true, although to a less degree, for the hyphae that attack the other internal organs of the insect.

The glandular tissues such as the silk glands and the malpighian tubules are the next structures to be attacked, and these also soon become filled with the fungous mycelium. There is some evidence that these structures may be attacked more rapidly when the larvae are inoculated by injecting a suspension of fungous spores into the alimentary canal. By the time the fungus has permeated the glandular structures, it is usually found to be commencing the invasion of the remaining tissues of the larvae, such as the chitinous lining of the hind- and fore-intestine (Pl. VIII, Fig. 9). Moreover, as the mycelium of the fungus has often been observed in the tracheae of the insect, the process of respiration must be greatly impaired by

<sup>1</sup> I wish to take this opportunity of thanking Mr. M. F. Crowell, for kindly allowing the use of this drawing of the European corn borer.

this invasion (Pl. VIII, Fig. 10). The last structures to be attacked by the fungus are the muscles (Pl. VIII, Fig. 10), the nervous system, and gonads (Pl. VIII, Fig. 7). In one instance, the gonads were observed to be infected,



TEXT-FIG. 2. A sagittal section through the body wall of a corn-borer larva showing an early stage of infection in which the fungus has penetrated the chitinous layer and reached the internal structures, causing a pronounced disintegration of the outer portion of the chitin as shown at the right. At the left the laminated, sclerotized layer of chitin is seen to be but little invaded.  $\times 900$ .

but then only after the parasite had invaded practically all of the other tissues of the host. The nervous system is attacked more readily than the muscle tissues, but in both of these the cells are not destroyed and do not disintegrate nearly so readily as do those of the fat bodies and glandular structures. The muscles seem to be the most resistant to the attacks of the fungus, this being especially true of the longitudinal muscles along the body wall of the larvae. The writer has often observed that when the various other body tissues show the presence of abundant fungous mycelium, the muscles apparently remain free from fungous invasion. In no case were resting spores found in the tissues of the European corn borer, nor have any ever been found on artificial media.

It is difficult to conceive how any means of penetration other than that in which invasion is preceded by enzymic action could apply here, since the chitinous layer is so thick that it would seem impossible for a fungous hypha to penetrate by mechanical means alone. When the fungus penetrates the body wall of the insect from the surface to the interior of the host, enzymic action seems to play the greater part. On the other hand

when the fungus is piercing the chitinous layer from the interior of the insect to the exterior of the host, there seems to be a great deal of mechanical pressure exerted as the diameters of the hyphae are greatly decreased when they are forcing their way through the hard striated chitin (Pl. VIII, Fig. 8). Also, when the hyphae are about to burst through the outer layer of the chitin, there is a definite swelling of the hyphae which would lead one to conclude that the action is mechanical to some extent at least. A section illustrating this point is shown in Pl. VIII, Fig. 5, where a definite swelling may be seen in the hypha which is pushing the pigment granule of the chitin outward.

In the various publications on the parasitism of entomogenous fungi, there is frequently an assumption that the fungus penetrates directly through the skin of the insect, and many investigators seem to be of the opinion that fungous spores are unable to germinate and cause infection by way of the alimentary canal. Unfortunately, no one has published illustrations to support these views, and it is, of course, almost impossible to demonstrate this without the use of sections. Burnside (2), however, working on diseases of the honey bee, reports that in the case of infection by *Aspergillus flavus*, only a few larvae are attacked as a result of direct penetration of the skin by germinating conidia; whereas infection of adult bees with this and other pathogenic fungi usually results only from the germination of spores within the alimentary canal.

In *B. Bassiana*, not only is there abundant indication that the chitin may be penetrated directly, but also there is good evidence to show that infection may take place by way of the alimentary canal, for, as has been shown above, when larvae are inoculated by anal injection or spores, they contract the disease.

#### EXPERIMENTS ON MANCHURIAN CORN-BORER LARVAE TO ASCERTAIN METHOD OF INFECTION.

During the course of this investigation large numbers of corn-borer larvae were imported from Manchuria, and when they were submitted to temperatures and moisture conditions which hastened the emergence of insect parasites, a high percentage of these larvae died due to infection by *B. Bassiana*. Since these imported larvae contained insect parasites which were later to be liberated and distributed in the United States for the purpose of controlling the corn borer, it was a serious loss to have so many of them die before the parasites could be secured. As it was not known whether the larvae were infected before being shipped to this country, nor whether the fungus was on the outside of the larvae or in a dormant state internally, the writer thought it advisable to make a histological study of these larvae to settle the question.

Since the larvae were not received by the writer until late in the spring of 1932, only a short time was available in which to investigate this phase; hence the following report will be a review of the preliminary observations only.

It was found that when the larvae imported from Manchuria were kept in a cool place, having a temperature range from  $10^{\circ}$  to  $14^{\circ}$  C. or below, the fungus did not develop and the larvae appeared to be perfectly healthy. The larvae which were kept at room temperature, however, soon showed signs of being diseased, and usually about 95 per cent. died within a period of ten days. In order to learn if this high mortality might be prevented, sixty larvae taken at random from a sample lot of 500 that had been kept in an ice chest at  $10^{\circ}$  C. were treated as follows: twenty of the sixty were immersed successively in three separate dishes of 70 per cent. alcohol, and were allowed to struggle and wriggle about in each dish so that any spores lodged on the surface of the skin might be washed off. These were then passed through three separate dishes of sterile distilled water and transferred to sterile Petri dishes, containing moist filter paper. Twenty other larvae were immersed in alcohol baths, and were then dipped in a 1 per cent. solution of mercuric chloride. The remaining twenty were kept untreated as controls. The next day there was one dead larva in each dish, and the treated larvae continued to die at the same rate as the untreated. At the end of ten days one larva was alive in the control and in the one treated with alcohol, while two were alive in the lot treated with mercuric chloride. Since the larvae continued to die after they had been immersed in alcohol and passed through three changes of sterile distilled water, it seems that infection must have been internal. This experiment was repeated on three different occasions, using larvae from different sample lots, and in each case the same results were obtained. In order to ascertain further the portal of entry of the fungus, twenty of the diseased larvae were sectioned, and in no case did the position of the hyphae indicate that they had penetrated the body wall from the outside. This fact would seem to support the above contention: namely, that the infection is internal, and therefore the fungus must be present in a dormant condition in these larvae shipped from Manchuria as long as they remain in a cool place (below  $14^{\circ}$  C.), but when submitted to high temperatures and humidities, the fungus takes on an active roll, develops, emerges, and eventually kills the larvae. All these larvae were killed as soon as they were noted to be diseased, and upon sectioning it was found that already the fungous hyphae had invaded all the various internal tissues of the insect; in no case were hyphae found in the body wall.

The progress of the disease in the Manchurian larvae when they are left in a moist chamber is similar to that already described for American forms. About twenty-four hours after the characteristic pink colour was

noted the larvae began to turn white as a result of the out-cropping of the fungous mycelium over the whole surface of the body. Again, it was noted that the hyphae within the tissues of the host had a much greater diameter than the mycelial strands that had already reached the chitin and were about to emerge on the body surface. Upon emerging from the body wall, a septum forms across the hypha, and this terminal cell thus formed becomes an oval-shaped conidium that is soon budded off (Pl. VIII, Fig. 6). Individual mycelial strands of *Beauveria* are capable of pushing through and emerging from the body wall of the larvae (Pl. VIII, Fig. 5), and do not show the tendency to clump or cluster together that Sawyer observed in the case of *Entomophthora sphaerosperma* parasitic on the black-headed fire worm of the cranberry. The fungous hyphae continue to grow out from the corn-borer larvae, and soon develop large numbers of conidia on zigzag phialides borne in whorls along the hyphal strands. These whorls or heads of conidium-bearing phialides increase in size and in number until the larvae are completely covered, thus giving them the powdery, mealy appearance already mentioned. These dry masses of conidia can then be readily blown about by the wind, thus disseminating abundant inoculum for further infection.

Although conidia have not actually been seen germinating in the alimentary canals of these Manchurian larvae, still the above experiments would seem to support the assumption that they may cause infection. Because of the small size of the conidia ( $1.5$  to  $2.5\mu$ ) and the presence of food particles in the digestive tract, it is very difficult to witness the initial stages of conidial germination. Also, by the time the disease is first evident, the fungus has developed to such an extent that it would be extremely difficult to find the specific place where infection originally occurred. It seems probable, therefore, that larvae imported from Manchuria are already infected before leaving that country, but the fungus remains dormant till these larvae are submitted to temperature and moisture conditions favouring the emergence of insect parasites which are to be distributed in this country in order to help control the European corn borer. As there seems to be no cure for the disease once the organism has gained access to its host, the only alternative seems to be to obtain larvae from some region other than Manchuria if those containing valuable insect parasites are to be secured.

*Infection experiment with the corn-borer pupae.*

It is obvious that if in the control of insect pests by entomogenous fungi the particular fungus in question can attack more than one stage in the life-history of the insect, greater significant control measures could be expected. Since the pupae of the corn borer are also ensconced within the succulent tissues of the corn plant where conditions ideal for fungal development

are present, it seemed advisable to find out just how *B. Bassiana* affected these pupae.

Five pupae were dusted with inoculum, placed in a moist chamber, and after the seventh day white masses of the fungus were seen to protrude from the thinner intersegmental spaces of the pupae. All five of these were sectioned, and it was found that the fungus could penetrate the body wall of the thinner intersegmental spaces of the pupae, but in no case were hyphae found in the thicker portion of the segments. After the fungus penetrates the pupal cases, the mycelium soon permeates all the various tissues of the pupae.

I wish to acknowledge my indebtedness to Professor William H. Weston, Jr., for his constant interest, and for many helpful suggestions and criticisms given during the course of this investigation. I also wish to thank Dr. K. A. Bartlett of the European Corn Borer Laboratory, Arlington, Mass., for supplying most of the larvae used in these experiments.

#### SUMMARY.

This paper presents first a brief life-history of the European corn borer under the conditions which prevail in the New England area of infestation, and then describes the development of *B. Bassiana* in corn-borer larvae inoculated on the body surface and in the alimentary canal.

The larvae were inoculated by allowing them to crawl over a culture of the fungus and also by injecting them with a spore suspension.

An effort was made to follow the development of mycelium from a limited amount of inoculum, but certain factors that could not be controlled made this impracticable.

Most of the infected larvae turned a characteristic pink colour, became dull and sluggish, and failed to respond readily to external stimuli. If kept in a moist atmosphere a white mycelial outgrowth enveloped the larvae and abundant sporulation took place. As the internal development of the fungus progressed, the larvae became mummified and brittle.

The germinating spores on the surface of the larvae produced infection hyphae which at any point except the head were observed to penetrate directly the thick sclerotized epidermal layer of the larvae, and also the thinner portions of the chitinous covering of the pupae. In addition, from the study of the Manchurian larvae, there were definite indications that infection may also take place by way of the alimentary tract.

After infection through either port of entry, the fat bodies were the first structures to become infested with the fungus, the glandular structures and ganglia were next affected, while the muscle tissue seemed to be the most resistant to fungus invasion.

It would seem therefore that *B. Bassiana* under favourable conditions

can readily penetrate the larvae and be a virulent parasite of the European corn borer. From studies made it seemed apparent that larvae imported from Manchuria have already been infected in their original home, and so succumb quickly to attacks of *Beauveria* when they are submitted to environmental conditions here that are favourable to the development of the fungus. Thus the Manchurian larvae apparently would not provide a favourable source of material for establishing insect parasites in this country to help control the European corn borer.

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#### EXPLANATION OF PLATE VIII.

Illustrating Professor C. L. Lefebvre's paper on 'Penetration and Development of the Fungus, *Beauveria Bassiana*, in the Tissues of the Corn Borer'.

#### PLATE VIII.

Photomicrographs of sagittal sections (7 microns thick) of parts of the European corn-borer larvae, showing the normal chitin and the early stages of infection.

Fig. 1. Sagittal section through a portion of the abdomen showing normal chitin with pigment granules in the surface layer, and fat cells below.  $\times 650$ .

Fig. 2. Sagittal section through a fold of the skin showing the fungus, after thirty-six hours, penetrating the chitin, but not yet invading the internal tissues of the host. The fat cells in the lower half of the figure are distinctly free from fungus invasion.  $\times 130$ .

Fig. 3. Sagittal section through a fold of the epidermis showing fungous spores (s), some of which have germinated and penetrated to the muscle tissue (M).  $\times 650$ .

Fig. 4. Sagittal section through a fold of the skin showing fungous hyphae which have penetrated the chitinous layer in large numbers and in some cases have grown into the fat cells at (F).  $\times 650$ .

Fig. 5. Sagittal section of body wall showing fungous hypha (H) growing out through the chitin, and forcing a pigment granule (P) from the sclerotized layer.  $\times 435$ .

Fig. 6. The hypha of *B. Bassiana* growing out through the chitin to the exterior of the larva, where, as shown at (s), ellipsoidal conidia are budded off.  $\times 435$ .

Fig. 7. Sagittal section through mid-portion of corn-borer larva showing the fungus permeating and destroying the fat cells (F), and also growing throughout the gonad (G), and in the mid-intestine (M.I.).  $\times 55$ .

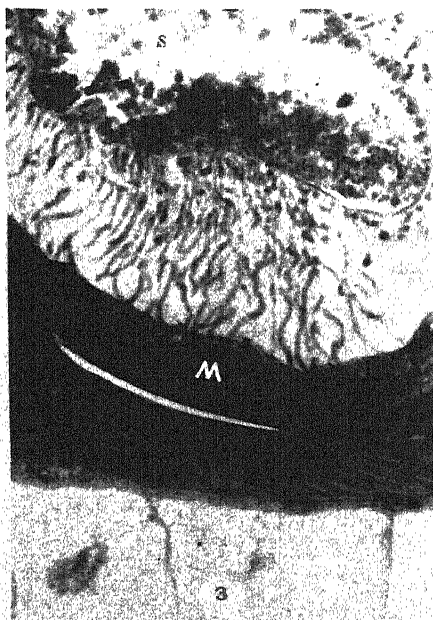
Fig. 8. Enlarged hyphae which have completely destroyed the fat cells (F). Note that the individual hyphae become thinner as they penetrate the hard chitinous layer (C).  $\times 435$ .

Fig. 9. Section through anterior portion of a larva showing the fungus penetrating the chitinous lining of the fore-intestine (I).  $\times 400$ .

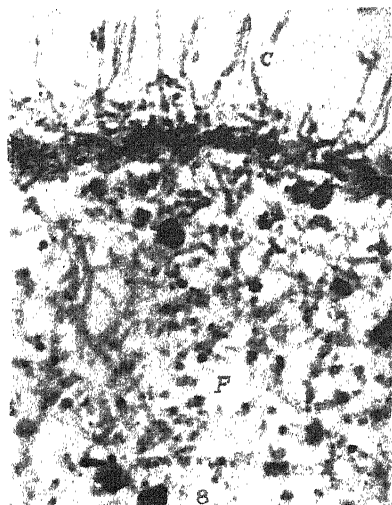
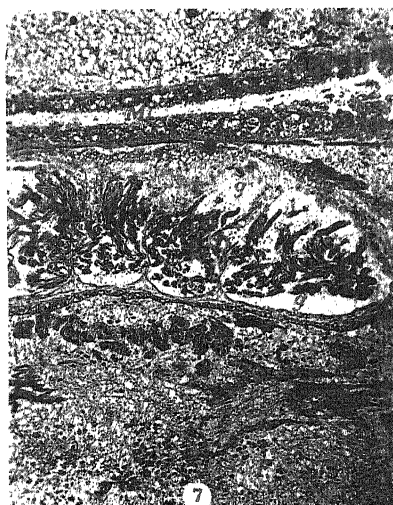
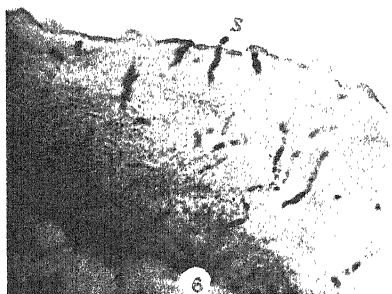
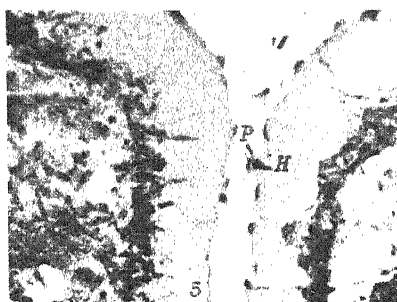
Fig. 10. Section of corn-borer larva showing, in cross-section, the tracheae (TR.) invaded by hyphae. The fungus also may be seen within the muscle tissue (M).  $\times 90$ .







LEFEBVRE — BEAUVERIA IN THE TISSUES OF THE CORN BORER.





# Spore Discharge in *Basidiobolus ranarum* Eidam.

BY

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With two Figures in the Text.

ALTHOUGH *Basidiobolus ranarum* has been studied by a number of mycologists, no one seems until recently to have disputed the statement made by Eidam (1) that the conidia are shot off from the conidiophores. Nowak (2) has now done so. He found in his cultures on a medium of unspecified composition, presumably synthetic, that the conidia were not discharged. Instead, the conidiophore broke down under the weight of the developing conidium, the latter dropped on the agar, sometimes rolled for a short distance with portions of the conidiophore attached to it, and eventually germinated. As these observations suggested that the conidia were not shot off, Nowak tried a further experiment. He supported a piece of agar at a distance of approximately 0.5 cm. above a mycelium bearing conidiophores, wrapped the culture in paper to exclude air currents, and left it undisturbed for a time. As he did not find conidia on the agar when he examined it, he concluded that this experiment afforded further evidence that the conidia of *Basidiobolus* are not violently discharged. Nowak says that his observations need confirmation. He admits the possibility that conidia may be shot off under natural conditions, but he considers it is more probable that the conidia of *Basidiobolus* are not shot off, and suggests that Eidam's work was done under the influence of an unsound comparison with such fungi as *Pilobolus* and *Empusa*. The marked disagreement between Nowak's opinions and those of others who have worked with *B. ranarum* led me to make a few simple experiments; the results may be of interest to botanists who have no personal acquaintance with the fungus.

The cultures used in the experiments were started by transferring small blocks of agar bearing non-fruiting mycelium of *B. ranarum*, careful choice of the inoculum ensuring that the cultures were quite free from conidia at, and immediately after, inoculation. Consequently, any conidia found subsequently in the cultures must have developed after the Petri dish was closed, and could not have been dropped during transfer.

During February and March 1933 fifty cultures were made on potato agar.<sup>1</sup> After inoculation the cultures were left undisturbed, at an average distance of six feet from a window, for three or four days. When the cultures were examined, all showed a few detached conidia lying around the inoculum, and many distributed on the bare agar over a triangular area stretching from the young mycelium towards the window. The densest accumulation of conidia occurred a little beyond the outermost tips of the spreading hyphae. A culture was chosen at random, and counts of the detached conidia were made along two lines at right angles, intersecting in the middle of the mycelium; one line passed through the centre of the triangular area, and so pointed towards the window. The results of these counts are given in Fig. 1.

The distribution of the conidia, and the distance at which some of them lay from the nearest hypha, gave little support to the view that they had rolled into position. Moreover, a film of liquid could be seen around the conidia on the agar, and the movement of such light objects on a wet surface seemed hardly possible. The possibility was, however, tested. In five cultures fields were selected, marked, and mapped. The dishes were then shaken, inverted, and inverted and shaken. After each operation the marked field was compared with the map. Indications were not obtained that the conidia were displaced by the violent treatment; they would move even less readily without assistance. Yet further evidence was desirable. Accordingly, a freshly inoculated culture was placed on a shelf in a window, and shielded so that light entered by a lateral aperture 2.5 cm. wide. The dish was tilted so that the surface of the agar rose towards the light at an angle of approximately 30°. Three days later the culture was examined. Detached conidia lay in plenty on the bare agar between the developing mycelium and the light, and none could be found elsewhere. If these conidia had rolled into position they had rolled up the slope, an unlikely event. The results of a count made along a line through the area occupied by the conidia appear in Fig. 2. The experiment was confirmed by two repeats.

The next step was to determine if conidia could pass vertically through a space above a mycelium; if this could occur, little doubt could remain that they had been projected from the conidiophores. One experiment will be reported in detail. At 11.10 a.m. on 6th March, 1933, a freshly inoculated culture, quite free from conidia, was covered by a lid over which black paper had been pasted, leaving a central hole 2.5 cm. in diameter. Beneath this hole, the under side of the lid was thinly smeared with paste. The culture was put at once into a dark room, and after sand had been drawn around the sides of the dish to stop any direct air currents, a 60-watt

<sup>1</sup> Potatoes, 250 grm. Boil for 1 hour, allow to settle, decant and filter the liquid and make up to 1 litre. Add 2 per cent. agar, autoclave at 30 lb. pressure for 20 minutes and pour hot.

bulb hanging 18 inches above the culture was turned on, and the fungus left to develop. The preparation was examined at 2 p.m. and at 5 p.m.; there was nothing to note. At 9.20 p.m., conidiophores were developing,

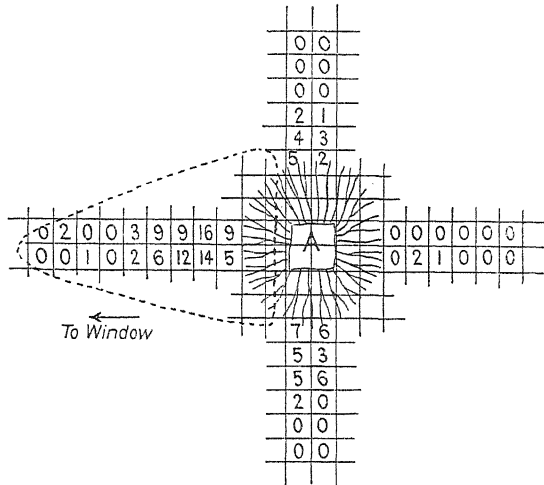


FIG. 1. Distribution of detached conidia of *B. ranarum* in a culture three days old, on potato agar. Conidia lay chiefly within the area indicated by dotted lines. A, inoculum and mycelium. Sides of squares, 2.5 mm.

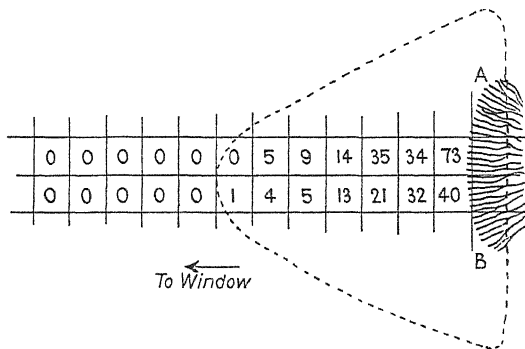


FIG. 2. Distribution of detached conidia of *B. ranarum* in a culture three days old, on potato agar. Conidia occurred only within the area indicated by dotted lines. Sides of squares, 2.5 mm. AB, outer edge of mycelium.

but conidia had not been shed. The light was left on all night. At 10 a.m. on 7th March, conidia, some already germinating, lay in abundance on the agar around the inoculum, and others had been caught by the paste on the lower surface of the lid; 36 were counted there in a circular area 1 cm. in diameter. At 3.30 p.m. the under side of the lid was cleaned, and a thin sheet of potato agar, taken from a freshly sterilized sample, was fastened to the lid underneath the hole in the black paper covering. The

distance from the fungus to the agar was about 1 cm. The culture was replaced under the light at 3.40 p.m. It was left until 9.35 p.m., when hundreds of conidia were found adhering to the agar; 104 were present on a circular area 1 cm. in diameter. This experiment was repeated three times, the repeats giving confirmatory results. Three more repeats, with the additional circumstance that stray light was somewhat cut off by placing a piece of brass tubing 15 cm. long vertically over the hole in the covering of the lid, gave results in agreement with those of the experiment reported in detail; the addition of the tube seemed to make no significant difference. It is clear from these experiments that the conidia of *Basidiobolus* can pass upwards to a height of at least 1 cm. above the mycelium, and it is reasonable to suppose that they are thrown upwards.

Numerous attempts were made to see conidia passing through the air. This was not seen, but the observations gave further evidence supporting the view that the conidia are shot off from the conidiophores. Some details follow. At 5.50 p.m. on 24th February, 1933, observations were begun through the lid of an unopened dish containing a mycelium four days old. A conidium, apparently mature, and still attached to its conidiophore, was found, and a note was made of any detached conidia lying on the agar within a circular area of 1 cm. radius around the foot of the conidiophore. At 5.55 p.m. there was a sudden jerk, the conidium and the conidiophore going at once out of sight. Focusing revealed the basal part of the conidiophore collapsed on the agar, but the conidium could not be found; it had disappeared from the area previously searched, for this still contained only the detached conidia previously noted. Nine other and similar observations were made by 6.15 p.m.; in all, the conidium went out of sight and could not be found. The observations were repeated on 2nd March with a mycelium five days old. Sixteen conidia disappeared from selected areas between 2 p.m. and 2.35 p.m. A conidium was never seen in flight; as soon as the jerk occurred, the conidium vanished. It did not fall directly to the agar, and underwent a lateral change of position of at least 1 cm.

Efforts were also made to see conidia come to rest on the agar at the end of their flight. For this purpose, cultures which had stood undisturbed for some days were moved to the stage of the microscope, and a light placed so that the conidiophores pointed towards it. Attention was then directed to areas on the agar where detached conidia were plentiful. Maps were made of the selected areas, and a watch maintained for arriving conidia. Although several hours on various days were devoted to these attempts, only one conidium furnished any evidence. It was not seen in motion, but suddenly appeared on the agar among the others already noted in that area. It did not roll.

The evidence gained from these simple experiments shows clearly that



when *B. ranarum* is grown on potato agar, the conidia undergo sudden and considerable changes in position, and the conidiophores simultaneously crumple and fall on the substratum. Although conidia were not seen passing through the air, it has been shown that they can be collected on a surface hung above a mycelium, and therefore that they do pass through the air. They must be projected from the conidiophores.

Nowak does not give the composition of the medium used in his experiments with the conidia of *Basidiobolus*, and it has not therefore been possible to repeat his work. The accuracy of his observations is not questioned here. It seems possible that the medium he used did not favour the development of robust and fully functional conidiophores. He may have obtained conidiophores resembling those developed from the secondary and tertiary conidia of *Basidiobolus*, which often collapse, but this is not the normal condition of the fungus. Nowak's suggestions cannot be accepted, and Eidam's original description holds good, at any rate for *B. ranarum* grown in artificial culture on potato agar.

The experiments were carried out in the Department of Botany, Birkbeck College, University of London.

#### SUMMARY.

Experiments are described which show that the conidia of *B. ranarum*, grown on potato agar, are shot off from the conidiophores.

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# On the Occurrence of Vessels in Selaginella.

BY

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With Plate IX.

HARVEY GIBSON (2) in 1894, produced evidence that in the genus *Selaginella* the tracheidal character of the xylem is the rule, but in two exceptional species, *S. rupestris* Spring, and *S. oregana* Eaton, both of them homophyllous forms, the metaxylem consists partly of scalariform vessels. He observed that this feature, occurring but rarely in true ferns, had not hitherto been shown to occur in the Lycopodiales.

During the course of previous work upon the sporangia of *Selaginella* (1), the author received herbarium material of several homophyllous species of this genus from Dr. W. R. Maxon of the United States National Museum.

The opportunity of extended observations upon the nature of the xylem elements in these homophyllous forms was thus provided, and in the present paper an account is given of the results of this investigation together with observations on the nature of the xylem elements in some of the heterophyllous members of the genus.

The herbarium material used in this work was very successfully resuscitated by the method described by McLean (4). After cutting into short lengths, of about 4 mm., the pieces were placed directly into absolute alcohol for twenty-four hours, then through graded alcohols into distilled water. Whilst in distilled water the specimens were left in the oven at 55° C. for two days. At the end of this time they were transferred to an aqueous solution of potash of a strength of 8 per cent. and left for a week at laboratory temperature. Neutralization was then effected by washing in weak acetic acid (15 per cent. of glacial acid), using several changes. After washing well in water the material was dehydrated, passed into chloroform and then embedded. In the case of fresh material it was always cut into short lengths before fixation. The fixatives used were medium chrom-acetic and Farmer's fluid (acetic-alcohol). Transverse and longitudinal microtome sections were cut at 4  $\mu$  to 10  $\mu$  in thickness.

The most effective stain for the pectic material of which the primary substance of the walls and the pit-closing membrane consists was found to be ruthenium red. A 0.02 per cent. aqueous solution of this stain was used and was made alkaline by adding 0.5 c.c. of concentrated ammonia to each 250 c.c.s. of the solution. Methylene blue was used as a counter-stain for the secondarily thickened lignified portions of the xylem walls. Good results were also obtained by using an aqueous solution of gentian violet with safranin as a counter-stain, and with Heidenhain's iron haematoxylin, also using safranin as a counter-stain.

*S. oregana* Eaton, was first investigated. Transverse sections of the stem xylem of this form supplied clear evidence of the middle lamella (Pl. IX, Fig. 1) stretching as a fine violet line, when stained with gentian violet, or a red line, when ruthenium red was employed, between the thickened portions of the primary wall at the angles of the elements. Longitudinal sections revealed the presence of the pit-closing membrane between the pits on the side walls of the elements, and the portion of primary wall, connecting together the two members of each pair of secondary bars. It is different, however, with the end walls. As may be seen from Pl. IX, Fig. 2, these walls are completely broken down, thus placing the elements in open communication with each other vertically. The positions of the remains of the end walls at the sides of the elements, Pl. IX, Fig. 2, *a-a*, *b-b*, show that in this species, at any rate, there was very little obliquity between the side walls and the original end walls. The latter were almost, if not quite, at right angles to the former. Evidence of a similar nature to that shown in Pl. IX, Fig. 2, was furnished by many parts of sections of different pieces of material of this form, making it clear that the xylem elements in *S. oregana* consist for the most part of true vessels. A really oblique wall with pits and pit-membranes was only found in one instance, so that tracheids also occur in this form but are evidently extremely rare. The pitting is of the scalariform type, there being usually one, sometimes two, rows of pits on the walls of the metaxylem elements.

In *S. rupestris* Spring, transverse sections of the stem xylem showed clearly the mid-lamella extending across between the thickened persistent portions of primary wall at the angles of the elements, in the space or slit between the secondarily thickened wall, exactly as in *S. oregana* (Pl. IX, Fig. 1).

Vertical sections showed the xylem structure to be of similar nature to that in *S. oregana*. The metaxylem elements showed one or two rows of scalariform pits in surface view, the end walls between the elements had disappeared, and their remains at the sides of the vessels indicated that in this species also these end walls were mostly at right angles to the vertical walls. In the pits on the vertical walls again the pit-closing membrane

could be distinguished and a few persistent oblique end walls were found with pits closed by pit-membranes, the xylem then consisting of tracheids as well as vessels.

*S. eremophila* Maxon, *S. densa* Rydb., and *S. Underwoodii* Hieron., showed structure of the xylem elements essentially similar to that which has been described for *S. rupestris*, as did also *S. arizonica* Maxon, except for the more numerous original end walls in the last species, that is, the metaxylem elements, before the disappearance of the end walls, had consisted of short, wide units. Pl. IX, Figs. 3 and 4 represent, in different foci, a vertical section of a portion of a large metaxylem element of this form. In Pl. IX, Fig. 3, the remains of the original end wall are shown by the projections on the side walls and in Pl. IX, Fig. 4, at a deeper focus, by a thickened rim, only part of which is in focus, which runs round the inside of the wall of the vessel. Pl. IX, Figs. 5 and 6 are photographs at different foci of the next end wall to that illustrated in Pl. IX, Figs. 3 and 4.

In the next three species the nature of the pitting on the metaxylem walls is different from that in the forms already dealt with. In each of the following cases the metaxylem elements bear a number of small oval pits, as many as seven in a series, in the case of the widest walls.

In *S. Hansenii* Hieron., the middle-lamella, extending across the small pit cavities, is plainly visible in transverse sections, giving the same kind of appearance in section of the wall as that figured at *a* in Pl. IX, Fig. 12, for *S. rupinicola*. The vertical sections showed that most of the elements were true vessels, as the end walls, which here were transversely orientated to the vertical walls, had been broken down, and their positions indicated by the projections at the side walls when the latter are seen in section (Pl. IX, Fig. 7, *a*), and by the thickened rims round the wall when the wall is focused in face view. Two of the latter are at a deeper focus and are faintly visible in this figure. One end of a pointed tracheid is also shown, but a better example is seen in Pl. IX, Fig. 8 at *a*, and in this figure the pit-closing membrane is seen on the walls *b*, *c*, and *d*.

Sections of *S. bigelowii* Underw., provided evidence of the pit-membrane in both transverse and longitudinal sections. The vertical sections also showed that, although most of the end walls had been at right angles to the vertical walls, indicated by the positions of their remains at the sides of the elements, yet some of the end walls were oblique and persistent, moreover the membranes in the pits on these oblique walls could always be distinguished. In this case also, then, the metaxylem elements consist of some tracheids with pointed ends as well as true vessels, where the whole of the original end wall between the elements has disappeared.

In the metaxylem of *S. rupinicola* Underw., typical pointed tracheids are very common, and it would seem that only rarely are true vessels formed. Pl. IX, Fig. 9 *a-a*, illustrates one of these cell-fusions, and in

the section figured in Pl. IX, Fig. 10, the cross wall has only partially gone, there being a broken line, stretching some way across the cavity of the element, which represents part of the primary wall, being stained as deeply with violet as the pit-membranes in the pits on the side walls, well shown at *b* in the same figure. In addition to the obliquely pointed tracheids in this form, there are many tracheids with rounded ends. A section of one of these tracheids is shown in Pl. IX, Fig. 11. In transverse section of the xylem these rounded end walls show reticulate markings (Pl. IX, Fig. 12). By reason of the concavo-convex form of this wall, it is at a deeper focus than the side walls in this figure. No evidence was gained of the pits in these rounded end walls being true perforations, there always being a membrane between the pits when seen in vertical section. A curious feature of these rounded end walls is that the secondarily thickened lignified bars appear to have been developed only on the concave side of the original primary wall (Pl. IX, Fig. 11).

The pit at *a* in Pl. IX, Fig. 11, is clearly perforated, but this is the result of penetration of the closing membrane by the fungal hypha which is seen to have penetrated the wall at this pit. Fungus mycelium with septate hyphae is present in and near this tracheid. An alternative explanation of this perforation could be that here is a transitional stage between a tracheid and a vessel, where some of the pits are perforated whilst others are closed, and the fungus had at this point grown through an open pit, but the absence of any evidence of true perforations in portions of material free from fungus would appear to indicate that this isolated instance of perforation was entirely the result of penetration of the closing membrane by the fungus.

As many as six or seven regular series of small oval pits are present on each facet of the larger metaxylem walls in this form.

An examination of the common European *S. spinosa* P.B., in many parts of both trailing and erect stem gave no evidence of the fusion of the xylem elements brought about by the disappearance of the end walls as was observed in the homophyllous species already described. In *S. spinosa* the only end walls found were in all cases oblique, with pits and pit-membranes intact, so that the xylem in this homophyllous form is entirely tracheidal. The pit-membrane is distinctly seen in both transverse (Pl. IX, Fig. 13) and longitudinal sections. Pl. IX, Fig. 14, illustrates the appearance in vertical section of the stem xylem in the ascending portion of the axis. The end of a sharply pointed tracheid is shown, and the pit-closing membranes are visible in the pits both on the vertical side walls and the oblique end wall. The metaxylem consists almost entirely of sharply-pointed scalariform tracheids, a few elements only being seen with simple pits reticulately arranged on the wall. The tracheid at *a* in Pl. IX, Fig. 14, shows pitting of the latter type, but the pits are at a deeper focus and are

shown only faintly in this figure. The xylem in the trailing part of the stem presents the same features.

Five heterophyllous species were examined. *S. chrysorhizos* Spring, *S. chrysocaulos* Spring, *S. pallidissima* Spring, *S. Victoriae* Moore, and *S. grandis* Moore. The pit-closing membrane was seen in all cases in both transverse and vertical sections. There was no evidence of the type of vessel found in the homophyllous forms examined. The xylem elements of each of these five heterophyllous species consisted entirely of pointed tracheids. The pitting of the metaxylem walls was of the same type in *S. chrysorhizos*, *S. chrysocaulos*, and *S. Victoriae*. In each of these three species scalariform pitting was the rule, but some of the larger tracheids had two, three, or four rows of pits in the form of narrow slits, on the wider walls. In *S. pallidissima* and in *S. grandis*, scalariform pitting was again the main type, but two, three, or four regular rows of small oval pits were characteristic of the widest walls.

Apart from *S. spinosa*, the xylem elements of the homophyllous species examined consist mostly of vessels, though some tracheids are present also. A further qualification of this statement is necessary for the case of *S. rupinicola*, where vessels do occur, but only rarely, the xylem for the most part consisting of tracheids with obliquely pointed ends and some tracheids with rounded ends.

Harvey Gibson's observation that vessels occur in the xylem of *S. rupestris* and *S. oregana* is confirmed, and, the occurrence of vessels is demonstrated in seven additional homophyllous forms. In the heterophyllous species investigated, the xylem is entirely tracheidal, as it is also in the outstanding homophyllous form *S. spinosa*.

With regard to *S. spinosa* it was pointed out by Harvey Gibson (2) that its stem anatomy differed entirely from that of the monostelic members of the genus, and in particular, furnished no evidence of close relationship to such types as *S. rupestris* and *S. oregana*, near to which it is placed by systematists. This distinction is true also for the rest of the homophyllous forms described in this paper, although each of them is monostelic, the stelar structure approaches more nearly to the single ribbon-shaped stele of the *Martensii* type, which is characteristic of the majority of the heterophyllous species of the genus, and is entirely different from the solid cylindrical stele of *S. spinosa*. The present work indicates the further distinction, that in the nature of its xylem elements *S. spinosa* is quite different from the other homophyllous forms investigated.

It is a point of interest and possibly of some significance that in *S. spinosa* and in the heterophyllous forms the individual tracheids are very much inclined to the lateral walls. On the other hand, the tracheids which occur in the other homophyllous forms are not so sharply pointed and, in addition, the latter possess vessels whose original end walls were

very little inclined, or else were quite at right angles to the vertical walls. There is the possibility that this slight inclination, or absence of inclination, of the end walls to the side walls, is favourable to the disappearance of the end walls. Indeed, the suggestion has been advanced by Halft (3) that the occasional occurrence of vessels in vascular cryptogams is found when the transverse and side walls of the xylem elements are not set at a sharp angle.

For the material I am indebted to Dr. W. R. Maxon of the United States National Museum, Professor Kashyap of the Government College, Lahore, and the Director of the Royal Botanic Gardens, Kew. The work has been assisted by the use of a microscope obtained by means of a grant from the Dixon Fund of the University of London.

#### SUMMARY.

In *S. oregana* Eaton, *S. rupestris* Spring, *S. eremophila* Maxon, *S. densa* Rydb., *S. Underwoodii* Hieron., *S. arizonica* Maxon, *S. Hansenii* Hieron., and *S. bigelowii* Underw., the xylem consists mostly of vessels, though some pointed tracheids also occur.

In *S. rupinicola* Underw., true vessels are rarely present, the xylem for the most part being made up of pointed tracheids, as well as some with rounded ends.

In *S. spinosa* P.B., the xylem is entirely tracheidal in nature.

The above species are all homophyllous.

In the heterophyllous species, *S. chrysorhizos* Spring, *S. chrysocaulos* Spring, *S. pallidissima* Spring, *S. Victoriae* Moore, and *S. grandis* Moore, the xylem is also entirely tracheidal.

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#### EXPLANATION OF PLATE IX.

Illustrating Dr. H. Duerden's Paper 'On the Occurrence of Vessels in *Selaginella*.'

(All figures are from untouched photographs.)

Fig. 1. *S. oregana*. Transverse section of the stem xylem showing the middle lamella in several walls. Gentian violet and safranin.  $\times 100$ .

Fig. 2. *S. oregana*. Longitudinal section of the stem xylem, showing at *a-a*, *b-b*, the positions of the original end walls. Ruthenium red and methylene blue.  $\times 100$ .



Fig. 3. Longitudinal section of a portion of a vessel of *S. arizonica* showing that the original end wall has disappeared except for the remains shown as slight projections on the side walls of the vessel. Gentian violet and safranin.  $\times 810$ .

Fig. 4. *S. arizonica*. The same piece of xylem wall as that shown in Fig. 3, but photographed at a deeper focus to show the remains of the original end wall as a thickened rim on the wall of the vessel. Gentian violet and safranin.  $\times 810$ .

Figs. 5 and 6 are two photographs at different foci of the same piece of xylem wall in *S. arizonica*, illustrating the appearance of the other end of the same vessel segment as that shown in figs. 3 and 4. The original end wall is gone, and its position indicated by the projections on the walls when the latter are seen in section, (fig. 5), and by a thickened rim when the wall is seen in face view (fig. 6). This thickened rim can also be seen faintly at a deeper focus in fig. 5. Gentian violet and safranin.  $\times 810$ .

Fig. 7. *S. Hanseni*. The stem xylem in longitudinal section. The ends of two vessel segments are seen and the end of a pointed tracheid. Ruthenium red and methylene blue.  $\times 1090$ .

Fig. 8. *S. Hanseni*. Longitudinal section of the stem xylem. The end of a pointed tracheid is shown at *a*. The pit-closing membrane is seen on walls *b*, *c*, and *d*. Ruthenium red and methylene blue.  $\times 1090$ .

Fig. 9. Vertical section of part of a vessel in *S. rupinicola*, the end of vessel segments with the cross-wall gone. The pit-membranes are clearly visible in the pits on the side wall. Gentian violet and safranin.  $\times 1090$ .

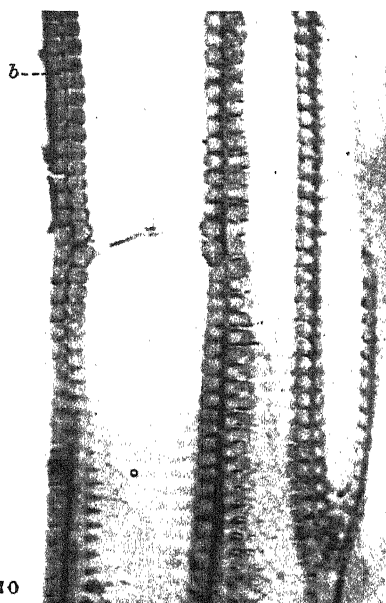
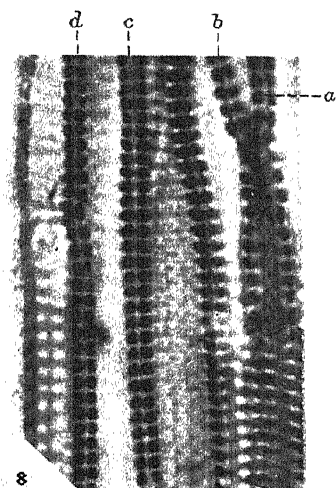
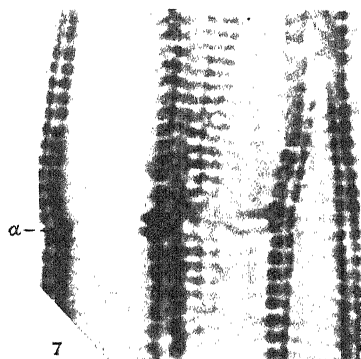
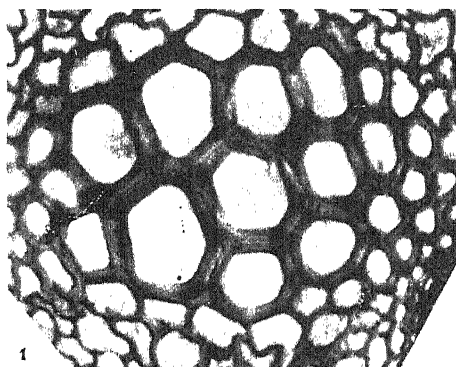
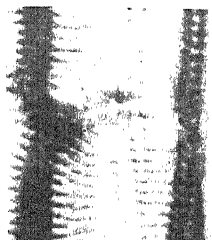
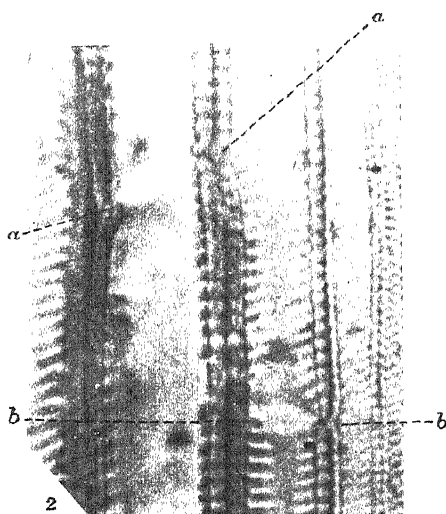
Fig. 10. *S. rupinicola*. Longitudinal section of the stem xylem showing the remains of an end wall stretching partially across the cavity of the element. The closing membrane is shown clearly in the pits on the side wall. The end of a pointed tracheid is also seen in this figure. Gentian violet and safranin.  $\times 1090$ .

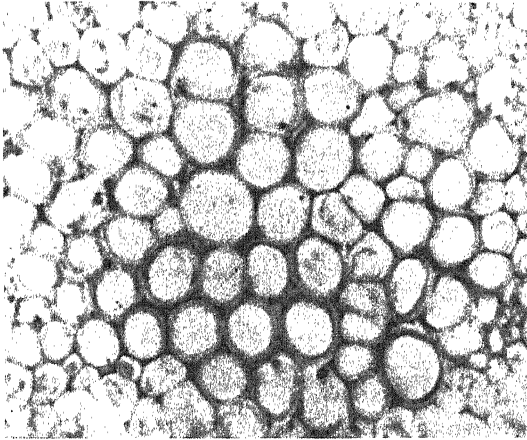
Fig. 11. Section of a tracheid with rounded end wall in *S. rupinicola* showing the pit-membrane in the pits except at *a*, which has been pierced by a fungal hypha. The secondarily thickened bars have been developed only on the concave side of the original primary wall. Small oval pits in several series are shown in this figure. Gentian violet and safranin.  $\times 1090$ .

Fig. 12. Transverse section of the stem xylem in *S. rupinicola* showing a reticulate end wall. The middle lamella is clearly shown in some of the walls (*a*). Gentian violet and safranin.  $\times 1090$ .

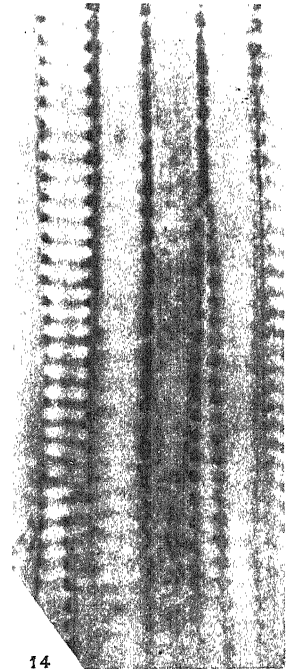
Fig. 13. *S. spinosa*. Transverse section of the xylem in the ascending axis showing the middle lamella. Gentian violet and safranin.  $\times 1090$ .

Fig. 14. Longitudinal section of the xylem of *S. spinosa* in the ascending axis. The end of a sharply pointed tracheid is shown. The pit-closing membrane is also seen distinctly on the tracheid walls. Reticulate pitting shown on wall at *a*. Gentian violet and safranin.  $\times 1090$ .



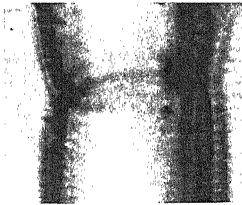


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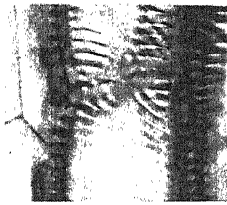


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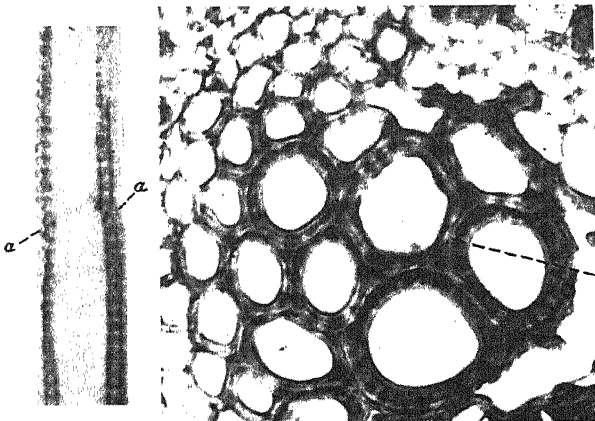
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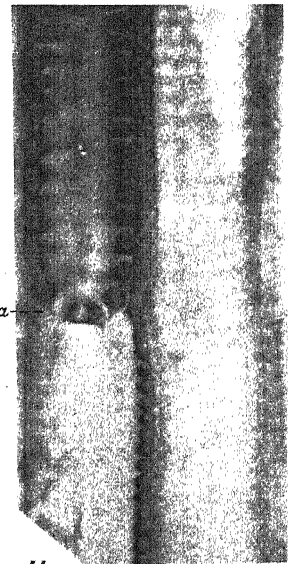


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# Studies in Growth and Differentiation.

## V. Histological and Metabolic Changes during Wound-Healing in *Kleinia Articulata* Haw.

BY

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With eight Diagrams in the Text.

### I. INTRODUCTION.

THE material of *Kleinia articulata* Haw. used in the examination of the problems discussed in these 'Studies in Growth and Differentiation' has necessitated extensive propagation of the plant by means of stem cuttings, several thousands having been made in the course of the work. Incidental to other anatomical studies, it was felt that the anatomical changes occurring at the wounded surfaces of the cuttings would yield interesting data regarding the development of a periderm in a stem that is largely composed of parenchymatous tissue. The observations of Thoday and Evans (11, 12) on the behaviour of solutes in the tissues of the plant led to an extension of the anatomical study to cover certain microchemical aspects of wound-healing. In particular it was felt that additional light might be thrown on the role of calcium malate and whether it could be mobilized in the plant-tissues.

Investigations of the wound-healing of injured plant-tissues have largely been made on the tubers of potato and sweet potato, in both of which the sequence of events leading to the establishment of a continuous sheet of periderm has been followed by several workers. The plant-tissues investigated are largely parenchymatous and, in general, three stages can be distinguished before a wound is completely healed:

1. A preliminary blocking of the surface by a fatty deposit. Free access of oxygen is required for this initial stage.
2. The formation of a phellogen beneath the blocked surface and its division to form cork initials.
3. The suberization of the cork initials.

Several observers, commencing with Kny (6) and Massart (7), showed that the appearance of phellogen was preceded by disappearance of starch from the cell layers among which the phellogen originates. Priestley and Woffenden (10) claim that the starch is converted anacrobically into fatty acids.

Artschwager (1) observes that protein is not removed. Nakano (8) describes a removal of oil from the wounded surfaces of *Ricinus* seedlings.

The length of time for complete cicatrization of wounds on a green plant varies with relation to the temperature and humidity conditions. Suberization is more rapid when wounded potatoes are kept in a moist atmosphere. The wound cork develops most rapidly when the atmosphere is almost saturated. Low temperatures similarly retard each stage of the process of wound-healing.

The wound-healing of succulent plants has received comparatively little attention, although their preponderance of parenchyma makes them suitable subjects for study. Miss Coutant (3) has described the sequence of periderm formation of two Arizona species of *Opuntia* in which conspicuous features are massive cork development, 18 cells or more deep, and production of phelloderm. No mention is made, however, of an initial blocking of the surface. Lignified walls, as well as suberized walls, are found in the periderm. An interesting feature is the occurrence of a second meristem below the phellogen, where it is first detected in the phelloderm, after three to four weeks. It produces short tracheides parallel to the surface. Gertz (4) has made observations on two *Kleinia* species, and states that in *K. neriifolia* Haw. groups of lignified stone cells and short tracheides occur in the periderm.

The present account is largely devoted to observations on the healing of stems of another *Kleinia* species, *K. articulata* Haw. In addition to the examination of the stages leading to the permanent formation of wound-cork, a study has been made, day by day, of the migration of selected inorganic salts and organic substances by microchemical methods. A description of the anatomy of this species will be found in an earlier paper in this series (18).

#### MATERIAL AND METHODS.

*The material.* Three series of experiments, I, II, and III, have been made. In I, plants, well established in soil, were used, the stems cut transversely, and the stumps left to heal. In II, fresh cuttings were made of segments of cylindrical stems so that two wound-surfaces were available, the lower surface being buried in sand. Series I and II were made during the period of active vegetative growth (January to March) and carried on for several weeks. A number of plants in each series had to be discarded as they had rotted at the base, and the next batch of plants were grown

under more suitable conditions of aeration and drainage. Series III was commenced at the close of the growing season; cuttings similar to II were used, except that the basal cut surface rested on sand and a layer of finely broken brick was poured over the sand to support the stumps in an erect position without being in contact with waterlogged sand. All the experimental plants were kept in a cool greenhouse, series II and III rather drier and warmer than series I. Only in the first series were the moisture conditions such that an average humidity of 95 per cent. was obtained. The average temperature was low compared with the temperatures quoted by workers on potato and sweet potato.

Sections were cut by hand or with hand microtome at right angles to the wounded surface, and examined without previous fixation, as, in addition to histological observations, microchemical data were collected of the distribution of calcium, potassium, magnesium, calcium oxalate, free oxalic acid, phosphate, and inulin. The methods described by Thoday and Evans (11, 12) for microchemical localization were employed:

calcium	by precipitation with alcoholic oxalic acid;
potassium	„ „ „ „ platinic chloride.

This method was checked by a method used in the microchemical examination of blood described by Breh and Gaebler (2), in which potassium is precipitated as silver-potassium-cobaltinitrite, which is more insoluble than the sodium potassium cobaltinitrite usually employed:

oxalic acid	by precipitation with strontium sulphate;
magnesium	„ „ microcosmic salt;
phosphate	„ „ ammonium molybdate;
inulin	„ „ strong alcohol.

For histological preparations sections were stained in Sudan III, Nile blue sulphate, and, later, ammoniacal gentian violet, following Tison's recommendation (14).

#### HISTOLOGICAL CHANGES.

In all cases, immediately after injury the surface was bathed with sap exuding from the parenchyma cells and also from the resin canals. An aromatic odour was noticed for a short time, aromatic oils evaporating from the resin canals. The surface remains moist and glistening for several hours, and basal surfaces in contact with damp sand only dry off completely after a period of four days. Table I shows an appreciable time-lag in the later stages of periderm formation behind the corresponding stages in the aerial lesions. The exudation contains traces of phosphate and more minute traces of potassium as indicated by microchemical tests applied to the exudate in fine capillary tubes. After 24 hours, the cortex and pith

surfaces become concave due to the collapse of the injured cells and the contraction and death of adjacent cells (compare Coutant (3), p. 356); this concavity is more marked across the wider core of pith than across the narrow ring of cortex (Diagram, A 2), but in the latter the contraction is sufficient to pull inwards the collenchyma and the epidermis. The vascular bundles thus project above the two concavities. After 48 hours the aerial surface is usually dry and the outer two to three layers have become impregnated with fatty acids, their walls staining deep blue with the Nile blue sulphate; some fat is also present, as flecks of red can be detected. This impregnation is first visible in the outermost layer of intact cells over the cortex and spreads inwards over the pith.

The concave surfaces tend to deepen up to the sixth day, and the ends of the vascular bundles are discoloured. At the end of a week the impregnation of fatty acids in the surface layers is complete, and this blocking skin presents a distinct glistening surface, staining uniformly in the Nile blue sulphate without any trace of red. Division walls can now be seen in cells in the second or third layer below the blocking sheet in the immediate neighbourhood of the vascular bundles (bundle zone). The phellogen spreads slowly across the cortex and pith, often two or three days elapsing before it is continuous beneath the lesion. At the same time the glistening skin becomes faint brown and the cells between it and the phellogen lose their contents. The phellogen is not laid down, however, in the epidermal or collenchymatized sub-epidermal layers if the wound has been made across an old stem.

In the second week after injury, the phellogen has cut off a single layer of cork initials the walls of which are only lightly stained by Nile blue sulphate. This original layer of cork cells becomes more strongly impregnated during the following week, but it is only rarely that any other layer of cork initials becomes suberized. There may be five to ten layers of unsuberized cork initials.

Artschwager (1) notes that newly formed periderm in potato does not give the characteristic suberin stain with ammoniacal gentian violet. Herklots (5) has shown that, after blocking of a wound is complete, the phellogen is most active in acid media, but no suberization of its daughter-cells will take place unless the tissue is transferred to a neutral or an alkaline medium.

About the eighteenth day the pith surface begins to lose its bowl-shaped appearance and phelloderm cells are then being cut off from the cork meristem. Anthocyanin can often be detected in the phellogen and phelloderm for several days, imparting a blue suffusion to the healing stump; this is an evanescent feature, no anthocyanin being found after 30 days. The surface is once more flat in the third week after injury and, after longer periods (over 30 days), it may become convex. About the



fourth week of healing, cell division begins in the pith below the phelloderm extending over several rows of cells. This may be comparable to callus formation, except that it is so long delayed (Diagram, A 4). No corresponding divisions occur in the cortex. The lesion is now completely healed, and subsequent changes are of minor histological importance. The most important is the 'collenchymatization' of 2-3 layers of phelloderm cells, in which calcium oxalate crystals are deposited as the walls show the first stages of thickening. This tissue closely resembles the hypodermal collenchyma of the mature stem.

The basal injuries follow a similar sequence over a longer period. The delay in forming a suberized skin is accompanied by a more extensive collapse of pith cells, so that the concavity becomes very deep and irregular. Occasionally rotting of the stump prevents complete healing: such rotting is progressive and causes death of the whole cutting within a short time. When the corky tissue is laid down it rarely presents a tight drum-skin surface, but is wrinkled or studded with small bulges. Fissuring across the pith cylinder is also a common feature in the bottom half of a stump. In series III such fissures were found at all levels between the top and bottom periderms in nearly every plant examined. Adventitious roots usually emerge from three weeks onwards after planting, and, after considerable periods, bases of rooted stumps show a prominent, convex, cork surface while the inner limit of the phelloderm is concave, so that the thick pad of periderm takes the form of a biconvex lens. Callus activity, though late in starting, is often conspicuous in basal injuries after several months. 'Collenchymatization' of two or three layers of phelloderm may also take place, but after a period of four to five months their crystals of calcium oxalate are yet only minute and sparse.

In *K. articulata* wounded surfaces thickly coated with a wax mixture showed no blocking film or trace of suberization within a period of observation of ten days. Nor, however, was any phellogen produced, so that phellogen formation was at least retarded. An additional feature of interest is that the pith, instead of collapsing, becomes slightly convex and glistening, though the cortex collapses as usual.

Longitudinal incisions by means of an ophthalmic lancet were made at various points along leafy branches, thus exposing a smaller wounded surface than the transverse amputations. Slits near the apex broadened deeply from a V-shape to a U-shape; the nearer leaves and petioles showed a temporary infusion of anthocyanin on the lower surface. These leaves were not wilted. Browning of the surface and the first appearance of wound-cork was appreciably delayed to 21-35 days. Incisions in more mature parts of the branch, wherever they were made, gave shallower concavities which became filled with scar tissue after 2-3 weeks. The periderm was first seen on the edges of the cut a week after injury.

The complete cicatrice thus comprises :

- (a) A thick deposit of fatty material.
- (b) One or two layers of empty cells, often unsuberized.
- (c) One or two layers of suberized cork.
- (d) Several layers of unsuberized phellem.
- (e) The phellogen.
- (f) Several layers of collenchymatous phelloderm containing calcium oxalate. These layers are continuous and similar to the hypodermis.
- (g) Parenchymatous phelloderm merging into
- (h) a band of callus derived from the pith parenchyma.

TABLE I.

*Time Sequence of Changes at Surface.*

(Figures are days from time of cutting.)

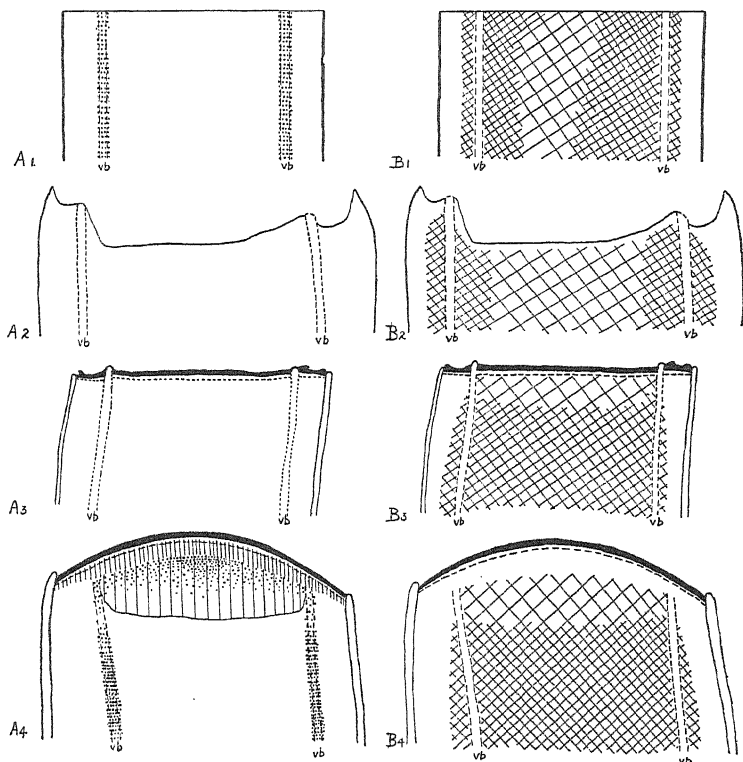
Series.	Duration of Observations.	Surface.	Size of cutting.	Surface dry.	Surface completely blocked.	Phellogen first detected.	Phellogen continuous.	Suberization of cork initials.	Collenchyma.	Callus formation detected.
	days.		c.m.							
1	63	Top	5.0	—	5	6	9	18	32	—
2	105	Top	2.5 & 5.0	2	5	11	16	20	—	22
3	57	Top	5.0	1	1-4	4-7	11	39	—	—
2	118	Base	5.0	5	20 (partial)	—	20	—	c. 100	31
2	38	Base	2.5	6	16 (partial)	—	20	—	—	—
	57	Base	5.0	4	11	7	14	39	—	—

#### METABOLIC CHANGES.

It has already been pointed out that a depletion of starch precedes phellogen initiation and activity in those plants where starch is the principal storage material. A similar loss of starch is reported for *Opuntia* (Coutant (3)), where, also, calcium oxalate crystals gradually enlarge in size from the third day after injury until they are very large by the twenty-fourth day. *Kleinia articulata* has no starch accumulation in any tissue, but depletion of other substances has been established, prior to the initiation of phellogen activity. The normal distribution of calcium, phosphate, and other ions has been described in earlier papers in these studies (Thoday and Evans (11, 12)). It will be noticed in what follows that there is a close correspondence between the upper and lower wounds in the time sequence of changes following injury.

*Calcium* (Diagrams B 1, 2, 3, 4). Little change in calcium distribution is seen for two or three days after injury, but about the fourth day there is

a gradual withdrawal from the intact cells below the wound extending for 2-3 layers in depth. As they become totally exhausted of calcium, lower layers in turn commence to lose it, and often the calcium oxalate pre-



DIAGRAMS A 1-B 4. Diagrams of sections representative of different stages in wound-healing. A 1, 2, 3, 4. Showing phosphate distribution by stippling. B 1, 2, 3, 4. Showing calcium distribution by cross-hatching. 1. Initial condition. 2. Four days. 3. Twenty-one days. 4. Thirty-six days after cutting. vb. Vascular bundle. Phellogen shown by broken line under surface: blocking layer of dead cells, black; In A 4 close vertical shading indicates periderm, the spaced vertical lines indicate the region in which the pith cells have grown and divided. Each pair of diagrams, A 1 and B 1, &c. represent sections from the same stem.

cipitate, obtained with alcoholic oxalic acid, shows a gradual diminution from a dense grey, where calcium has normal abundance, to the clear tissue from which it has been removed.

The phellogen arises in cells from which calcium has been completely exhausted or are rapidly approaching that point. The living cork initials cut off from it never contain calcium (calcium malate) even in long-healed wounds (e.g. over 100 days), while the phelloderm and cells in the neighbourhood of the phelloderm, including the callus, show little free calcium. No calcium malate is found in the phellogen or beyond it in the cork.

When transverse fissuring of the pith has been found, as in series III, the cells lose their calcium a short time before their rupture.

Accumulation of calcium in portions of cuttings remote from the wound has not been detected, and there is no appreciable difference in calcium distribution between the upper and lower wound-surfaces of cuttings (see Table I). As already mentioned, the rhombohedral crystals of calcium oxalate appear in the phelloderm collenchyma after a month and grow to a large size.

*Potassium.* This does not show such a marked change, although a slight decrease in the density of the crystals of potassium chloroplatinate may be noted for a day or so after wounding. Potassium seems to increase in amount towards the base of a wounded stem, and in time it becomes abundant in all parts, including the phellogen and the cork initials. It has been detected in the latter three weeks after injury. A week or two after wounding a band of cells, including the phellogen, contains potassium more abundantly than the cells on either side. In individual plants, where potassium was not detected in pith cells when the experiment was started, it encroaches into the pith in this periderm band about the eighth day and then spreads downwards (away from the phellogen).

The pith was rich in potassium to a depth of 5–6 cells after 18 days; and to a depth of 1 cm. after 28 days.

Other species of *Kleinia* and allied genera examined are normally without potassium in sufficient concentration to be detected by the platinic chloride method, but in *K. neriifolia*, where it is present in small concentration in the stem, no potassium is found for at least a month after injury, and is only found very sparsely below the lesion after several months: no potassium has been found in the periderm. This is a marked difference in the wound reactions of the two species.

*Phosphate* (Diagrams A 1, 2, 3, 4). The effect of wounding is most striking in the distribution of phosphate, which is normally present along the cylinder of vascular bundles (bundle zone). Within 24 hours, probably within the first 12 hours, phosphate disappears entirely; not only from the cells close to the wound, but also for considerable distances. It has not been found in the exudation from the cut surface. In series III the basal ends showed more phosphate than the upper ends, and, after a period of three weeks, phosphate was still present immediately above the basal periderm but none for several millimetres below the upper periderm. Short stumps, less than 2.5 cm. long, are often totally depleted of free phosphate; they usually die before recovery.

After the phellogen has been active for a few days a small patch of phosphate appears, but not in the depleted bundle zone. This patch lies immediately below the phellogen in the centre of the pith and gradually extends radially and longitudinally. Especially in basal wounds several phelloderm layers may accumulate phosphate over a long period (from 50 to 100 days). It should be remembered that intact stems rich in phosphate show no localization of phosphate in the central pith (Thoday and Evans,

11, p. 790), and only one record of a trace of phosphate in such a position has been made, apart from these lesion patches. After 30 days some phosphate reappears in the bundle zone, but there is always a broad gap below the periderm where phosphate does not re-establish its former distribution or density.

*Magnesium* is found normally in the central pith. After 24 hours very slight traces only could be found in cells up to ten layers from the injury, the magnesium having been removed from the cells below the wounded region. At the end of the week it can only be detected with great difficulty in a few cells, but after a long interval (50–60 days) magnesium accumulates once more to the base of the phelloderm. It is mainly localized in the central pith, but a lesser amount has been traced along the bundle and in the inner cortex.

*Calcium oxalate*. The young stem of *K. articulata* shows no deposition of calcium oxalate until after the hypodermal layers have become collenchymatous. Examination of a long branch at intervals shows that, with the increase in diameter of the stem from tip to base, there is a corresponding increase in the thickness of the cuticle, the number and size of calcium oxalate crystals, number of rows of collenchyma cells, and the thickness of their walls (Table II; and see 13, p. 675, Text-fig. 2).

TABLE II.

Distance from Apex, mm.	Circumference mm.	Cuticle Thickness. $\mu$	Calcium oxalate crystals. Number per mm.	Length of side in micrometer divisions. <sup>1</sup>	Collenchyma. Rows.	Thickness of wall.	
< 1.0	7.28	< 2	none	—	—	—	
27.2	9.15	< 2	none	—	2	+	
61.3	16.17	2.5	none	—	2	+	
67.5	13.75	2.5	none	—	2	+	
72.5	19.28	2.5	8	2 to 4	3	++	
81.5	24.02	2.5	33	2 to 4	3	++	
91.1	23.86	2.5	37	2 to 4	3	++	
97.9	24.08	{ 2.5 to 5.0	33	3 to 4.5	3	++	
106.1	27.91	5.0	25	3 to 5	3	+++	
118.1	25.15	5.0	54	4 to 6	3	+++	in 1st col. layer
—	—	—	25	—	—	—	in 2nd col. layer
133.1	26.78	5.0	71	4 to 6	3	+++	in 1st col. layer
—	—	—	25	—	—	—	in 2nd col. layer
154.6	31.27	5-7.5	54	4 to 6	—	—	

When cuttings are made, the amount and size of crystals will vary according to the size of the cutting and the distance from the apex (representing the age at that level). Series I was sufficiently young to have no calcium oxalate present; it began to accumulate in the hypodermis, in the

<sup>1</sup> 1 div. = 0.0024 mm.

second week after injury, near the wound, and within a few days small crystals were seen at all levels. These crystals had attained their maximum size by the end of the fourth week, by which time the second and third collenchyma layers also contained calcium oxalate. It did not appear in the phelloderm till the fifth week.

In the early stages of wound-healing a small amount of free oxalic acid occurs and can be precipitated as strontium oxalate. It has been detected during the first week in the hypodermal cells (compare 12, and, later, in the region where calcium oxalate crystals are to appear in the periderm. Free oxalic acid cannot be detected in either situation after the calcium oxalate crystals begin to form.

*Inulin.* Young active shoots do not show a marked accumulation of inulin for several weeks. In Series I, where such shoots were used, little or no inulin could be traced at the commencement of the experimental period, and none appeared until the third week. It was then conspicuous, especially in the phelloderm, decreasing in amount in relation to distance from the lesion. Later there was some accumulation in the outer pith cells below the periderm.

*Reducing Sugars.* Miss Marion Roberts has shown that reducing sugars are normally absent from young shoots and have only been detected in cuttings after 60 days, thereafter increasing steadily in amount. The sugar is present in the cortex and bundle zone in close relation to basal periderms.

The principal microchemical data are summarized in Table III.

TABLE III.

(Figures are days from time of cutting.)

	Series I.	Top.	Series II.		Series III.	
	Top.		5 cm. Base.	2.5 cm. Base.	Top.	Base.
Ca. depletion commences	6-7	4	2	4	4	4
Phosphate depletion commences	2	1	1	1	1-2	1-2
Phosphate accumulation commences	12	—	12	12	11	11
Mg. depletion commences	1	—	—	—	—	—
Mg. accumulation commences	50-60	—	—	—	—	—
Inulin accumulation commences	22-23	—	—	—	—	—

#### WOUND-HEALING IN RHIZOMES.

The rhizome produces a normal periderm after several weeks' growth (13). A healed transverse wound, some months after injury, showed much fissuring of the pith reaching almost to the floor of the wound periderm, which was then 10-12 layers in thickness. The surface over the pith was convex, but the cortical wound cork was still concave. No phelloderm had been produced.

Calcium was absent from the periderm, but was abundant in tissues reaching to the phellogen, and was especially rich in the cortex. No calcium oxalate was found in the sub-phellogen layers, a feature correlated with the absence of collenchyma. Potassium was relatively sparse but distributed evenly throughout all tissues. Phosphate was only seen in the bundle zone up to the level of the periderm, and transverse-sections revealed that it was localized around the vascular bundles, not extending across the medullary ray parenchyma. Some inulin was present near the wound-periderm in the neighbourhood of the bundles, but not elsewhere within the centimetre length examined.

#### WOUND-HEALING IN LEAVES.

The leaves of *K. articulata* often show wound-healing though only a thin layer of scar tissue is found.

The cut surfaces dry off and their edges darken within a few days, a faint brown skin first appearing along the upper epidermis. This stage presumably corresponds with Wylie's 'pseudocicatrization' (15); the same shrinkage of cells adjoining the wound, pulling in the epidermal cells, has been observed. These cells are not suberized. In transverse injuries across the lamina only a few dividing cells are found, scattered beneath the collapsed cells of the cut surface; but injuries parallel to the main vein usually produce two to three continuous layers under the whole surface. No dividing cells have been observed below the cut end of a petiole 21 days after injury.

#### OTHER SPECIES.

Observations on the stages of healing in other species of *Kleinia* and closely related genera are now in progress, and, in general, show features parallel to those described above. Potassium is sparse in *K. neriifolia* and absent in other species examined, but calcium and phosphate are present in all the plants. Both show rapid depletion within a day or so after the injury, and in no case have they been traced in the tissues formed by the phellogen. Wound lesions of *K. neriifolia* are complex, for, in addition to a vigorous development of cork, a vascular meristem produces short tracheides below the floor of the periderm, and groups of stone cells are found scattered in the periderm (Gertz, 4). The development of the tracheides and periderm is now being studied.

#### GENERAL REMARKS.

Although starch is not found in the tissues of *Kleinia*, yet it has been shown that the stages in wound-healing are accompanied by movements of certain (selected) ions away from the cut surface, similar to the depletion

of starch already recorded for other plants. A few preliminary observations on the movement of these ions have been made on injured potato tubers, and they indicate that phellogen activity is preceded, as in *K. articulata*, by a complex series of biochemical changes in the cells in the neighbourhood of the lesion. Potassium, sparse in the tuber, is withdrawn from the neighbourhood of a wound, but only over a depth of two to three layers. Phosphate is very abundant in the intact tuber, principally in relation to the vascular bundles. After injury there is a rapid removal of phosphate from the parenchyma, which becomes depleted of it by the end of the week. On the other hand, phosphate remains abundant in the immediate neighbourhood of tracheides to the edge of the cut over the same period. Phosphate is not precipitated in any cells where phellogen activity is manifest.

The migration of calcium and phosphate, in particular, is found in all the species of *Kleinia*, &c. so far examined, and their depletion commences immediately after injury.

Priestley and Woffenden, in their observations on wound-healing in the potato, maintain that phellogen activity is preceded by an increased sap flow *towards* the blocked surface: thus (9, p. 255), 'This blocking then appears to react upon the tissues within through the accumulation in the walls and intercellular spaces of a sap which provokes the activity of a meristem'; and (10, p. 115), 'The essential factors promoting the activity of this phellogen appear to be the accumulation behind the blocked surface of the sap containing substances diffusing from the vascular bundles and the production of an acid reaction just below the blocked surface by the anaerobic conversion of sugars into fatty acids.'

It is difficult to see how sap, a watery solution of a mixture of solutes, can ever *accumulate* in the walls, and there is no evidence to show that sap diffuses into the intercellular spaces. If sap passes into the cells for an indefinite period there is an obvious limitation to the volume of water that any cell can accommodate. It may be that the underlying idea is a raising of the concentration within the cell sap of the intact cells. Even so the microchemical data for *K. articulata* and other species clearly show that there is a removal of substances from the neighbourhood of the blocking membrane and not an accumulation, as Priestley and Woffenden suggest. Phellogen activity begins in cells that have lost their calcium malate, phosphate, and magnesium. The accumulation of phosphate and inulin observed in *K. articulata* only occurs after the periderm has been produced, and, so far, there is no indication that there is any accumulation of substances behind the blocking membrane prior to or during phellogen activity.

Thoday and Evans (12) show that potassium is distributed throughout most tissues near the apex of an actively growing shoot, but disappears from the pith with increasing age. Similarly (11) they show that the amounts of calcium and phosphate increase with the age of the stem, very



little being found close to the stem apex. It would appear that metabolism of the apical meristem is characterized by a uniform concentration of potassium and only traces of calcium and phosphate. After wounding, the localization and migration of these ions represents a return to this meristematic type of metabolism prior to the appearance of the phellogen. The tissues a little removed from the periderm soon regain the normal distribution, and the periderm is sharply defined microchemically from the subjacent tissues.

Phosphate is only found in the pith after wound-healing, and is then strictly limited to the central portion of the phelloderm and the neighbouring cells of the pith.

#### ACKNOWLEDGEMENTS.

The writer desires to express his best thanks to Professor D. Thoday for his continued advice and criticism during the course of this work; to Dr. H. Evans, who checked over some of the microchemical data, and to Miss Marian W. P. Roberts, whose work on species of *Kleinia* has afforded a source of useful comparison.

#### SUMMARY.

1. The sequence of changes following injury has been studied. In general they show agreement with the course of events described for potato, sweet potato, &c.
2. The exposed cells rapidly become impregnated with fatty materials which completely block over the injury.
3. A phellogen arises subsequently close to the fatty deposit and produces cork initials and phelloderm. Little suberization of the cork initials takes place, and some of the phelloderm layers become collenchymatous. This 'collenchymatization' is associated with deposition of calcium oxalate crystals.
4. Changes in mineral content have been followed in tissues beneath injuries. Wounding results in a rapid removal of calcium and phosphate, while potassium shows some slight depletion. Potassium soon regains its former abundance and distribution, and is found also evenly distributed through the new cells of the periderm.
5. Free phosphate and calcium are not found in the phellogen.

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# Studies in the Physiology of Wood-destroying Fungi.

## II. Temperature and Rate of Growth.

BY

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With Plate X and eight Figures in the Text.

### INTRODUCTION.

AMONG the factors influencing the growth of wood-destroying fungi, temperature is one of the most important; not only does it affect their rate of growth, and in consequence the rate of decay of the timber, but also it partly determines which shall be the predominant species in any locality.

An examination of the rate of growth of a number of the more important Basidiomycetes which grow on wood has been carried out in the course of a general study of the physiology of these fungi on which the authors are engaged.

A certain amount of work has been carried out on the temperature relations of wood-destroying fungi by various authors, but most of this is scattered throughout the detailed descriptions of the various fungi, and has not been used for comparative purposes.

Falck (3) determined the minimum, optimum, and maximum temperatures for several species of *Merulius* and *Lenzites*, and used these criteria as a means of distinguishing between nearly related species. He found that *Merulius sylvester*, a fungus closely resembling *M. lacrymans*, could be readily distinguished from the latter by its ability to grow at temperatures of 30°<sup>1</sup> and over. Similarly *L. saepiaria* and *L. trabea* were shown to possess markedly different cardinal points, enabling them to be separated on this character.

<sup>1</sup> N.B. Temperatures are given in degrees Centigrade except where otherwise stated.

Snell (12) determined the rates of growth of a number of wood-destroying fungi at various temperatures, and included the figures obtained in a key for distinguishing various species in culture. Liese (8) gives figures for the daily increment of plate cultures at laboratory temperature in his descriptions of a number of fungi.

Fritz (6) observed the growth of a number of wood-destroying fungi at various temperatures, and found that *Fomes igniarius*, *F. roseus*, and *F. fomentarius* all grew most rapidly at 30°, making but slow growth at 35°. She found the optimum for *P. schwewinitzii* to be 27°, with only very slight growth at 35°. 'Intensification of the colouring of the mat was brought about by increase of the heat in *F. igniarius*, *F. roseus*, and *L. saepiarum*, while *F. fomentarius* produced more delicate tints at the higher temperatures.' A temperature of 22° was chosen at which to grow the cultures for standard descriptions. Mounce (11) found that the rate of growth of *F. pinicola* is very slow at 6° to 8° and increases up to about 29°, above which it falls off, till at 35° it is practically inhibited.

In a recent paper Lindgren (9) compares the rates of growth of *L. saepiarum*, *Polystictus versicolor*, and *Lentinus tigrinus*, as measured by the Petri dish method, with the rate of decay brought about in different timbers at various temperatures by these fungi. His results are discussed below.

#### EXPERIMENTAL PROCEDURE.

All the measurements of rates of growth were made upon cultures grown on 2 per cent. Kepler's malt extract agar in 10 cm. Petri dishes. In the first experiments the amount of medium in each plate was measured exactly to 20 ml., but it was soon found that the depth of the medium did not noticeably affect the rate of growth; so in the later tests an amount only approximately equal to 20 ml. was poured into each dish. The freshly made up medium was autoclaved once to melt the agar, and was then aseptically poured directly into sterile Petri dishes by means of a syphon introduced into the medium-flask before sterilization. The plates were inoculated centrally soon after the medium had set, with small transplants about 4 mm. square, cut from actively growing plate cultures of the fungi, and placed with the aerial mycelium downwards.

As a general rule the dishes were placed in the various incubators maintained at the different temperatures, immediately after inoculation, but where it was particularly wished to study the growth of certain fungi at temperatures near the minimum or optimum, the plates were kept at 23° until growth had just started from the inoculum. This was done because it was found that certain species are so slow in starting growth that the medium, especially at the higher temperatures, tends to become

dried up and contaminated before a sufficient number of readings can be taken. It was not found that this short preliminary incubation at 23° had any influence on the subsequent rate of growth observed later at other temperatures, provided that growth was allowed to become constant before readings were taken. The diameter of the colony was measured daily at the same hour (except at temperatures below 5°, when readings were taken weekly). Measurements were made with a piece of graduated card placed on the underside of the dish, which was held up to the light. Provided the layer of medium was not too thick, it was found possible in most cases to measure the diameter quite easily to the nearest mm. In this way removal of the Petri dish lids and consequent contamination of the medium were avoided. Where the colony was not perfectly circular, measurements were made along two diameters at right angles and the results averaged.

A few fungi produce a 'halo' of submerged mycelium in advance of the aerial, which makes observation difficult: in such cases readings were taken across the diameter, both of the aerial mat and of the 'Halo'. It was found that though the rate of advance of the submerged and of the aerial mycelium might be different, yet the proportions between rates at different temperatures were the same for the submerged and for the aerial, so that in the results given below only the figures for the rate of advance of the aerial mycelium are plotted.

#### RATES OF GROWTH.

In every case the figures given under the temperatures following the name of the fungus are the average daily increments in mm. of the diameter of the colony, based upon the measurement of 5 plates.

#### GRAPH I.

##### *Merulius lacrymans* (Wulf.) Fr.

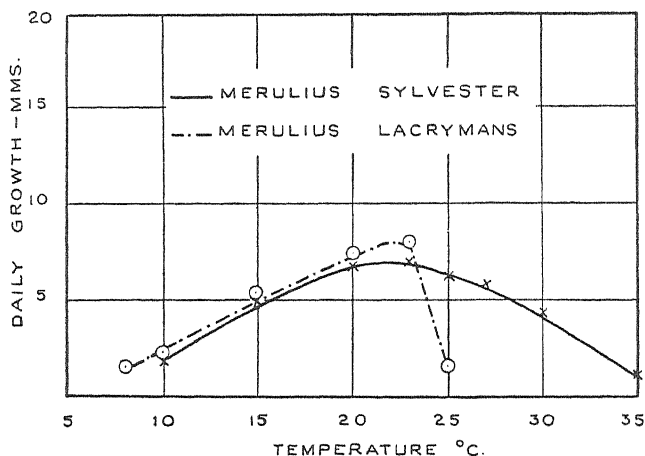
Temperature . . .	8°	10°	15°	20°	23°	25°	27°	30°
Av. daily increment	1.5	2.2	5.4	6.9	7.9	1.5	0	0 mm.

##### *Merulius sylvestris* Falck.

Temperature . . .	10°	15°	20°	23°	25°	27°	30°	35°
Av. daily increment	1.8	4.9	6.7	6.9	6.1	5.8	4.2	1.0 mm.

These two curves are of interest since they illustrate how the so-called 'wild race' of *Merulius lacrymans* may be readily distinguished from the *domesticus* variety which it closely resembles in culture. The rates of growth of these varieties are very similar up to 23°, but above that temperature the growth of *lacrymans* (*domesticus* Falck) rapidly drops off, ceasing entirely at 25-7°, while that of *sylvestris* continues up to about 36°. The very steep drop in the rate of growth of the *domesticus* variety when

the temperature exceeds about  $23^{\circ}$  is rather striking; not only is this an unusually low optimum temperature, but it is evident that this fungus is very sensitive to heat. The growth at temperatures above the optimum



TEXT-FIG. 1.

looks unhealthy and stunted, and the white mycelium develops patches of bright yellow, which are indicative of a check to growth. This intolerance of high temperatures is probably one of the reasons why *M. lacrymans* is so rarely found in the open upon timber which may become heated by sunlight. *M. lacrymans*, as far as is known, has never been recorded from a tropical country, and it is stated to cause much less damage to buildings in the United States of America, than it does in Northern Europe. This is no doubt partly due to the high summer temperatures and to the greater heating of buildings in the winter.

Though the growth of *M. lacrymans* is slow at low temperatures, this fungus can cause considerable damage in the timbering of cold stores maintained at temperatures a few degrees above freezing-point, and it is reported to be very prevalent in buildings in Russia.

GRAPH 2.

*Coniophora cerebella* Pers.

Temperature . . .	8°	14°	20°	23°	25°	27°	30°	35°	37°
Av. daily increment	1.5	4.3	8.6	10.4	10.0	7.8	2.9	—	0 mm.

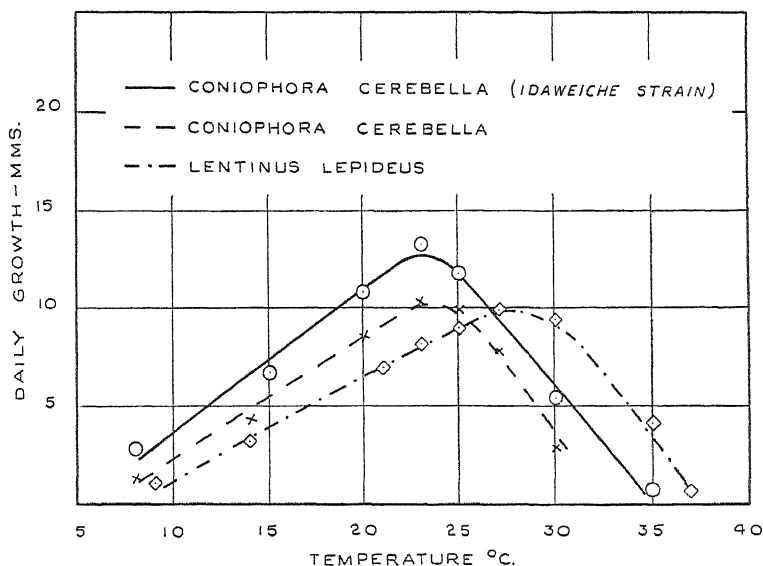
*C. cerebella* 'Idaweiche' form.

Temperature . . .	8°	14°	19°	23°	25°	27°	30°	35°	37°
Av. daily increment	2.9	6.8	10.8	13.3	11.8	—	5.5	0.8	0 mm.

*Lentinus lepideus* Fr.

Temperature . . .	9°	14°	21°	23°	25°	27°	30°	35°	37°
Av. daily increment	1.2	3.3	7.1	8.2	9.0	9.9	9.4	4.1	0.7 mm.

There are two well-marked strains of *Coniophora cerebella*, which can be readily distinguished in culture, one strain which forms a smooth, even mat of whitish, then brown colour, grows moderately quickly and the other,



TEXT-FIG. 2.

which has been called the Idaweiche form by Falck, grows more rapidly, and produces a thin yellowish then brown mat in which the strands stand out prominently. The difference in the appearance of the cultures of these two strains is so striking that it suggests that they are in fact different species, though in each case isolations from supposedly typical *cerebella* sporophores have given rise to both types of culture. The optimum temperature is about the same for both strains, and lies between 23° and 25°; this fungus is considerably less sensitive to heat than *Merulius lacrymans*, and continues to grow quite vigorously at 30° and over.

Curve 3<sup>1</sup> for *Lentinus lepideus* shows that this fungus has much higher cardinal points than *C. cerebella*, the optimum lying between 27° and 30°, and the maximum about 37°. This fungus occurs occasionally in railway-sleepers, paving-blocks, and poles which have been imperfectly creosoted. In these situations it must be exposed to comparatively high temperatures during sunny weather in summer. It also occurs in damp, warm, coal-mines. Snell (11) found approximately the same cardinal points as the above for this fungus.

<sup>1</sup> The curves are arranged upon the different graphs in such a way that the lines do not interfere and obscure each other, rather than for purposes of comparison.

GRAPH 3.

*Lenzites trabea*.

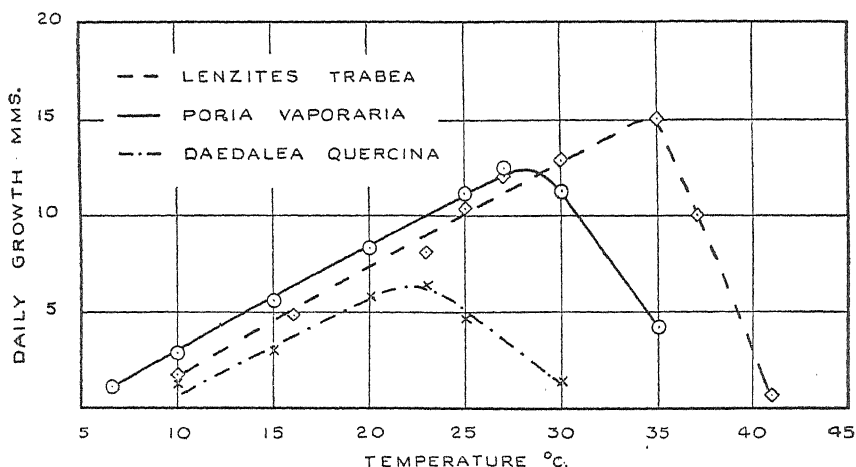
Temperature . . .	5°	10°	16°	20°	23°	25°	27°	30°	35°	37°	41°	44°
Av. daily increment	0	1.7	4.8	6.0	8.2	10.4	12.1	12.8	15.1	10.0	0.7	0 mm

*Poria vaporaria*.

Temperature . . .	5°	10°	15°	20°	23°	25°	27°	30°	35°	37°
Av. daily increment	1.1	2.2	5.6	8.4	—	11.2	12.5	11.2	4.2	0 mm.

*Daedalea quercina*.

Temperature . . .	10°	15°	20°	23°	25°	27°	30°
Av. daily increment	1.4	3.1	5.8	6.4	4.7	1.5	0 mm.



TEXT-FIG. 3.

*Lenzites trabea* (Pers.) Fr.

This fungus is characterized by its ability to grow at relatively high temperatures, the optimum lying about 35° and some growth taking place at 40°. Falck established a species called *L. thermophila* which he described as growing well at high temperatures: cultures of this fungus have been compared with those of *L. trabea*, and were found to be identical (2). *L. saepiaria* also grows well at high temperatures, but the optimum lies about 30°, and definitely lower than that of *L. trabea*.

*Poria vaporaria* (Pers.) Fr.

The strain of the fungus tested was obtained originally from the Baarn collection, and had been isolated by Professor Klujver. Similar cultures have been isolated by the authors both from the long pored, rather thick *Polyporus vaporarius* form, and from the thin, expanded, poroid form with strands of mycelium running off from the fruit body which is known as *Poria Vaillantii*. The growth curve may be compared



with that of *Merulius lacrymans*; the decay of timber caused by these two fungi is very similar, but their cultures can be readily distinguished, *P. vaporaria* being able to grow vigorously at 30° C.

*Daedalea quercina* (Linn.) Fr.

The growth of this fungus at some temperatures proved difficult to measure, as the aerial mycelium was surrounded by a halo of mycelium submerged in the medium. The fungus grows somewhat slowly in culture, and possesses an unusually low optimum.

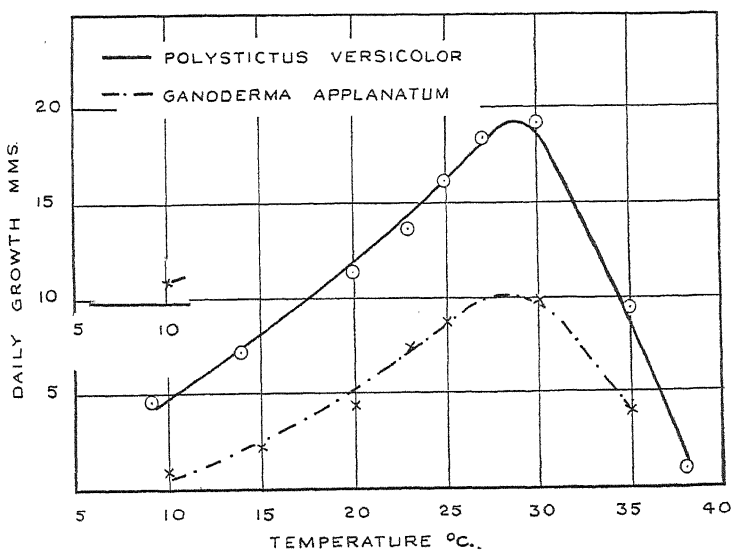
GRAPH 4.

*Polystictus versicolor*.

Temperature . . .	9°	14°	20°	23°	25°	27°	30°	35°	38°
Av. daily increment	4.6	7.1	11.2	13.5	16.0	18.3	19.1	9.4	0.8 mm.

*Ganoderma applanatum*.

Temperature . . .	10°	15°	20°	23°	25°	27°	30°	35°	38°
Av. daily increment	0.9	2.2	4.4	7.4	8.7	—	9.9	4.0	0 mm.



TEXT-FIG. 4.

*Polystictus versicolor* (Linn.) Fr.

This fungus, which is one of the most important agents of decay of Dicotyledonous wood in this country, grows rapidly in culture, and possesses a very wide temperature range with an optimum about 28–30°, which is similar to that found by Lindgren (9). The appearance of the mat of mycelium in a Petri dish culture varies greatly according to the

temperature. At 20° a young culture consists of a rather sparse adpressed mycelium with a loose cottony margin, and later a thin leathery skin-like mat is produced, while at 35° it forms a rather silky, somewhat branched, bushy mat, with much more initial development of aerial mycelium (see Plate X, Figs. 1 and 2).

*Ganoderma applanatum* (Pers.) Pat.

This fungus generally occurs in the heartwood of living Dicotyledonous trees, in which it produces a white rot. The temperature range is similar to that of *P. versicolor*—but the daily rate of growth in culture is about half.

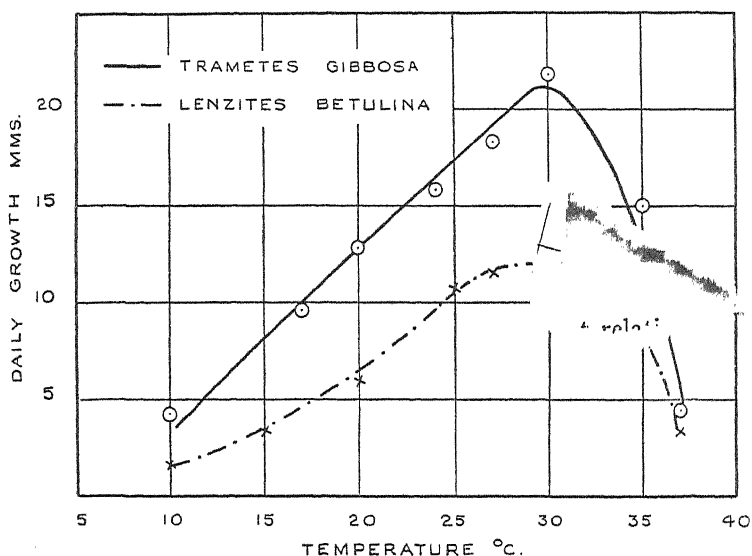
GRAPH 5.

*Trametes gibbosa*.

Temperature . . .	10°	17°	20°	24°	27°	30°	35°	37°	40°
Av. daily increment	4.2	9.6	12.8	15.8	18.3	21.8	15.0	4.4	0 mm.

*Lenzites betulina*.

Temperature . . .	10°	15°	20°	25°	27°	30°	35°	37°	40°
Av. daily increment	1.5	3.2	5.8	10.7	11.4	12.3	8.8	3.3	0 mm.



TEXT-FIG. 5.

*Trametes gibbosa*. (Pers.) Fr. and *Lenzites betulina* (Linn.) Fr. are two fungi which commonly occur on decayed Dicotyledonous timber. Occasionally forms may be found whose fertile surface is intermediate in character, and the identity of these specimens may be doubtful. It will be seen from the graphs that while the temperature range is wide and approximately

the same for both fungi, *T. gibbosa* grows about twice as fast in culture as does *L. betulina*.

GRAPH 6.

*Schizophyllum commune*.

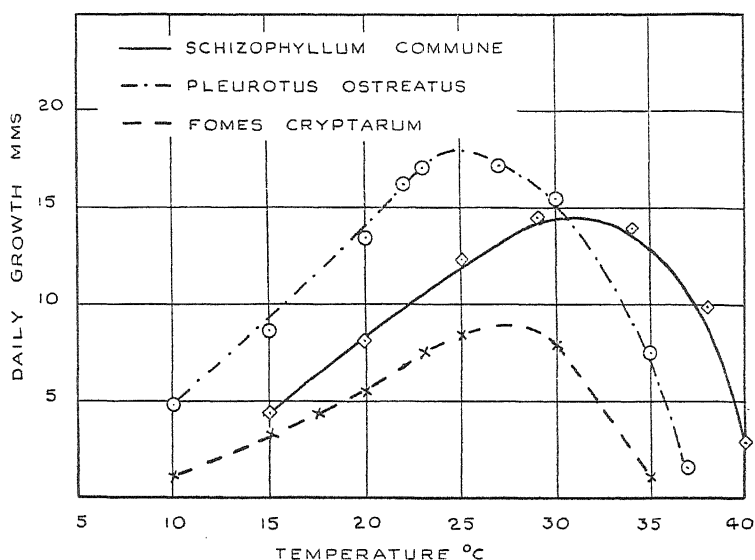
Temperature . . .	16°	20°	25°	29°	34°	40°	42°
Av. daily increment	4.4	8.1	12.2	14.4	13.9	9.9	mm., trace

*Pleurotus ostreatus*.

Temperature . . .	10°	15°	20°	22°	23°	27°	30°	35°	37°
Av. daily increment	4.8	8.6	13.3	16.2	17.0	17.2	15.4	7.4	1.6 mm.

*Fomes cryptarum*.

Temperature . . .	10°	15.5°	20°	23°	25°	30°	35°	37°
Av. daily increment	1.1	3.3	5.5	7.6	8.4	7.9	1.1	0 mm.



TEXT-FIG. 6.

*Schizophyllum commune* Fr.

This fungus is distinguished by the ability to grow well at surprisingly high temperatures. The optimum for growth is between 30° and 35°, and the maximum about 42–4° C. This fungus is of common occurrence in the tropics, and has been isolated on several occasions from tropical timbers, e.g. from *Shorea leprosula*, and from kiln-dried mahogany. It is not of economic importance, however, as it appears unable vigorously to attack the cell-walls of wood, and thereby cause any serious weakening or loss in weight of the timber.

*Pleurotus ostreatus* (Jacq.) Fr.

Possesses a wide temperature range with an optimum for growth of about 25° C. It is capable of making comparatively rapid growth at low

temperatures, the diameter of a culture at 10° showing an average daily increment of 5 mm.

*Fomes cryptarum*.

This fungus has been isolated on several occasions from decayed oak wood in buildings in this country. From the type of decay it causes, the appearance of the sterile sporophores, and the growth in culture, there is little doubt that it is identical with the fungus described by Mangin and Patouillard as *Phellinus cryptarum* Karst (10). This fungus is being further investigated by the authors. It may be noted that the plant in question is certainly not the *Fomes cryptarum* of some German workers who consider it a variety of *Fomes annosus*. The fungus grows well at 30°, but the optimum probably lies slightly below that temperature. Growth at low temperatures is very slow.

GRAPHS 7 AND 8.

*Stereum hirsutum* Fr.

Temperature .	3°	8°	16°	20°	25°	27°	30°	33°	35°
Av. daily increment	1.2	4.0	12.5	16.5	19.9	19.5	14.9	6.3	0.8 mm.

*Stereum purpureum* Fr.

Temperature .	3°	8°	16°	20°	25°	27°	30°	33°	35°
Av. daily increment	0.5	4.3	11.0	14.8	20.6	22.3	17.5	2.0	0.45 mm.

*Stereum spadiceum* Fr.

Temperature .	5°	10°	15°	20°	23°	25°	27°	30°	35°
Av. daily increment	0.5	3.1	5.7	8.4	10.2	11.4	9.0	2.9	0 mm.

*Stereum frustulosum* (Pers.) Fr.

Temperature .	10°	15°	20°	23°	25°	27°	30°	35°
Av. daily increment	0.6	1.1	2.0	2.8	3.2	2.9	2.1	1.2 mm.

*Stereum rugosum* (Pers.) Fr.

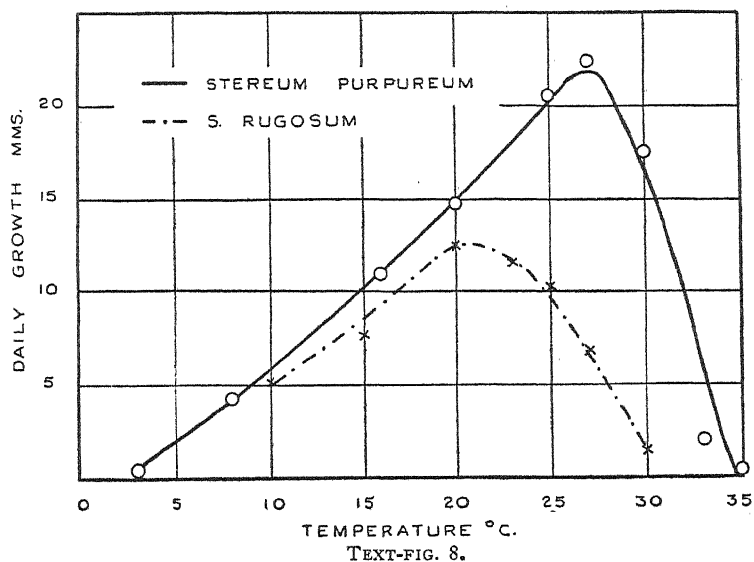
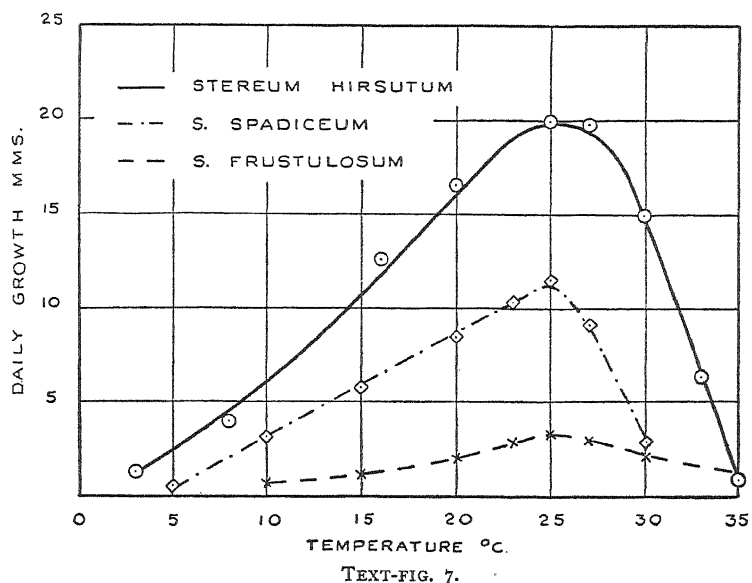
Temperature .	10°	15°	20°	23°	25°	27°	30°	35°
Av. daily increment	5.2	7.1	12.6	11.6	10.3	6.9	1.5	0 mm.

The temperature relations of the five species of *Stereum* which have been investigated are illustrated in graphs 7 and 8, and may be considered together. The following points may be noted.

1. With the exception of *S. rugosum* the optimum for all the species examined is 25–27°, and the range of temperatures over which they can grow is fairly wide.

2. At their optimum temperature *S. purpureum* and *S. hirsutum* grow more rapidly in culture than do any of the other fungi which were examined; they are also capable of making moderately rapid growth at the low temperatures, and their fruit bodies frequently appear during the winter months.

3. *S. frustulosum* grows extremely slowly in culture, but possesses a wider temperature range.



4. *S. rugosum* may be distinguished from the other species by having an optimum of about 23°.

Below are listed the rates of growth of a number of wood-destroying fungi, which have been previously investigated.

*Paxillus panuoides* Fr. (4).

Temperature .	9°	15°	20°	23°	25°	27°	30°	35°
Av. daily increment	1.2	2.2	3.6	4.0	3.9	3.6	3.4	0 mm.

*Trametes serialis* Fr. (1).

Temperature .	14°	25°	30°	35°
Av. daily increment	3.7	9.9	7.1	0.1 mm.

*Fomes annosus* Fr. (5).

Temperature .	8°	15.5°	20°	23°	25.5°	27°	28°	30.5°
Av. daily increment	2.2	8.5	11.8	13.0	11.0	9.3	7.4	1.0 mm.

*Polyporus adustus* Fr. (2).

Temperature .	15°	20°	25°	27°	30°	35°
Av. daily increment	2.8	7.9	8.0	7.8	3.5	0 mm.

*Polyporus funosus* (Pers.) Fr. (2).

Temperature .	15°	20°	25°	27°	30°	35°
Av. daily increment	2.5	5.1	7.4	6.4	1.5	0 mm.

## DISCUSSION.

The method used for comparing rates of growth at various temperatures was chosen, after some consideration, as being the simplest, and as one which can give rapid and repeatable results.

The absolute rate of growth as determined by these measurements has little significance, and cannot be directly applied to conditions of growth in wood. A fungus like *Stereum purpureum* which grows with great rapidity over malt agar, is able to decompose wood only very slowly. Then again the area of spread of the mycelium does not give a true measure of the total amount of mycelium present, but by this method the *relative* rates of growth of the same fungus at different temperatures may be determined.

Whether the relative rates of growth of fungi are exactly the same on wood as on agar media has not been definitely established. Lindgren (9) found that for both *Lenzites saepiararia* and *Polystictus versicolor*, the most rapid decay was caused at the temperature at which the fastest mycelial growth occurred, but that, on the other hand, *Lentinus tigrinus* brought about the greatest decay at 27° on certain timbers, while the most rapid mycelial growth took place at 32°. His results are somewhat inconclusive, as the losses in weight on which the estimate of the amount of decay was based were comparatively small, and the number of samples employed was not large, in view of the great irregularity which is always experienced in decay experiments with blocks of wood. He concludes that in all probability the assumption that the temperature relations of an organism on wood can be predicted from the results of tests on agar is sufficiently correct for all practical purposes. The drying out of wood blocks at the higher temperatures which may lead to a slowing down of the growth of the fungus is a factor which must be considered and controlled in any

experiments made to compare the rates of decay of blocks at different temperatures.

#### TEMPERATURE AND DISTRIBUTION.

Temperature is one of the most important ecological conditions affecting the distribution of fungi. The saprophytic fungus flora of the world is very much more uniform than that of the flowering plants, and many species of fungi have a world-wide distribution. Fungus spores are so easily distributed by air currents that some of the common species will be found growing wherever a supply of suitable food material and the necessary temperature and humidity conditions are present. It may almost be said that if suitable pabulum be exposed under the appropriate conditions, a certain series of saprophytic fungi will be certain to make their appearance, though the frequency with which any one species appears will depend upon the density of the local infection.

The temperature relations of a fungus largely determines its geographical range, some species possessing a wide temperature range may be found almost universally distributed. Occasionally a strain of a fungus isolated in the tropics has higher cardinal points than a strain of the same fungus isolated in a colder country.

The extremes of temperature experienced have obviously a limiting effect upon the species found in any locality; *Merulius lacrymans* (*domesticus*) is never found in the tropics (*vide* p. 484), nor is it ever found in a hot coal-mine where temperatures average over 25° C. Fungi which occur very generally distributed, such as *Polystictus versicolor* and *Pleurotus ostreatus*, are found to possess a wide temperature range.

The question as to whether one species or another becomes dominant in any environment where the conditions of food-supply and humidity are equally favourable to both, will largely depend upon the relative rates of growth at different temperatures. Supposing the spores of two wood-destroying fungi such as *Coniophora cerebella* and *Lentinus lepideus* are placed upon a medium equally suited to both; at 20° *Coniophora* is able to grow 50 per cent. faster than *Lentinus*, and this differential rate will soon give *Coniophora* an advantage enabling it to occupy most of the medium; on the other hand at 30°, since *Lentinus* can grow about twice as fast as *Coniophora*, the positions will be reversed. It is therefore evident that temperature plays a large part in determining what species are predominant in decaying any piece of timber.

#### USE OF TEMPERATURE CURVES.

The temperature and rate of growth curves are useful in several ways, e.g. for:

(1) characterizing certain species in culture enabling them to be recognized and distinguished from related species;

(2) indicating the possibility of any species occurring in a given locality or country;

(3) providing data as to the optimum conditions under which to carry out tests with the fungus of the resistance of any timber to decay, or of the toxicity of wood preservatives.

#### THERMAL DEATH POINTS.

In this paper no reference has been made as to the temperatures which are necessary to kill wood-destroying fungi. Experiments upon cultures in agar can give only a rough indication of the behaviour of the fungi in wood; those which are the most resistant in agar cultures will be among the most resistant in wood, but the actual times necessary to sterilize fungi growing in wood, particularly if this is at all dry, will be much longer than in agar cultures. Snell (13), using  $\frac{3}{4}$  in. blocks artificially inoculated with *Lenzites saepiaria*, *L. trabea*, *Lentinus lepideus*, *Trametes serialis*, and *T. carnea*, found that none of the fungi within these blocks was able to withstand 131° F. (55° C.) for twelve hours at moist heat, while it took 221° F. (105° C.) for twelve hours to kill all the fungi with dry heat.

Hubert (7) carried out a series of experiments upon infected timber which was kiln-seasoned according to the usual schedules, and found that, provided this treatment was carried out at temperatures exceeding 120° F. (49° C.), any fungi growing in the blocks were killed.

Liese (8) determined the times necessary to kill various wood-destroying fungi growing in agar cultures. Generally speaking his results, considered in relation to those recorded in this paper, show that the fungi which possess the lower optima are also those which are most sensitive to killing by heat, and that those which can grow at the higher temperatures are among the more resistant to heat.

A knowledge of the temperature relations may therefore be helpful in deciding what temperatures it is necessary to employ when steaming or kiln-drying timber infected with wood-destroying fungi.

#### SUMMARY.

The importance of temperature in determining not only the rate of growth of wood-destroying fungi, but also the actual species dominant in any locality is discussed.

The technique employed for measuring rates of growth of cultures on malt agar at various temperatures is described.

Tables and graphs illustrating the rates of growth of a number of wood-destroying fungi are given.

The usefulness of these graphs in assisting identification of the fungi in culture is discussed, and reference is made to the relation between the temperatures suitable for growth and the thermal death points of the fungi.



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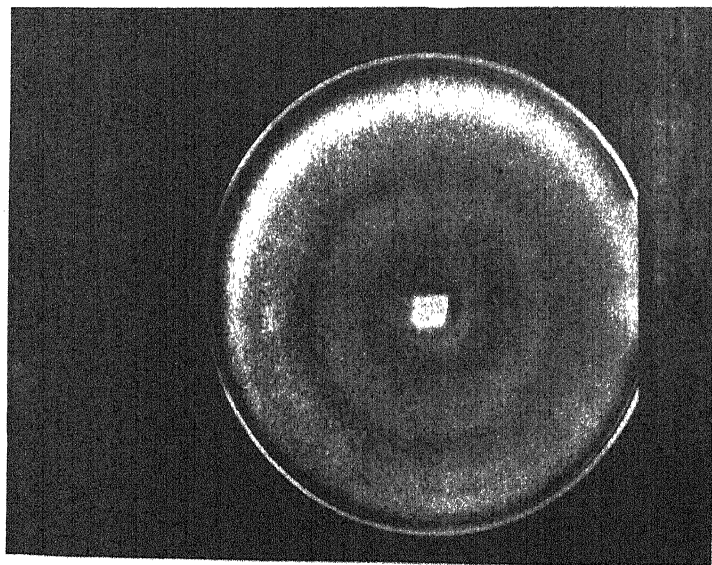
EXPLANATION OF PLATE X.

Illustrating Messrs. Cartwright and Findlay's paper on 'Studies in the Physiology of Wood-destroying Fungi. II. Temperature and Rate of Growth.'

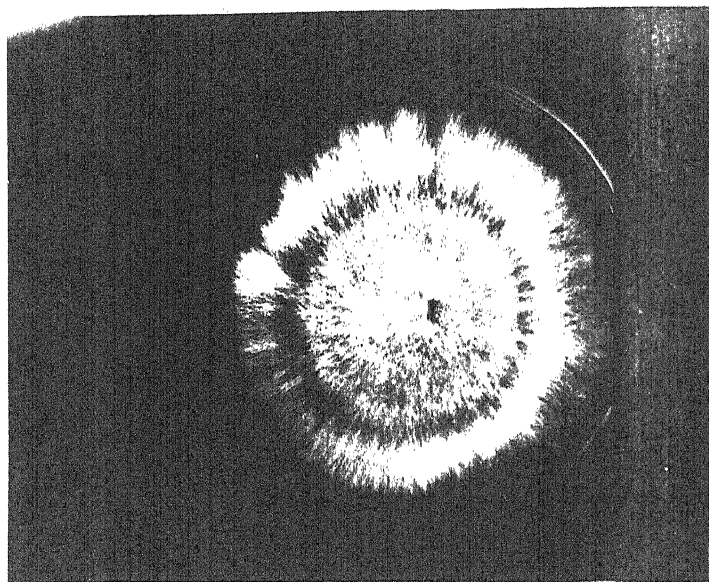
Fig. 1. Petri dish culture of *Polystictus versicolor* on 2 per cent. malt agar, grown at 20° C.

Fig. 2. Petri dish culture of *P. versicolor* on 2 per cent. malt agar, grown at 35° C.





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CARTWRIGHT & FINDLAY — TEMPERATURE AND GROWTH.

PLATE CXL



# On the Use of Simultaneous Observations on Successive Leaves for the Study of Physiological Change in Relation to Leaf Age.

BY

F. J. RICHARDS.

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With two Figures in the Text.

ACCOUNTS of research dealing with the influence of leaf age on physiological characteristics appear in botanical literature from time to time. The approach to this problem is frequently simplified by taking advantage of the fact that the leaves present on a shoot at any particular time constitute an age series, thus rendering the comparison of leaves of different ages an easy matter and conveniently reducing sampling errors to a minimum. In a general sense the succession of leaves does, of course, form a series of structures of increasing age, those nearest the growing point being the youngest and those farthest removed the oldest. Furthermore, since the order of death among the leaves on a shoot is in general the same as their order of production, the presumption is strong that other corresponding stages in development, if such exist, are reached by the various leaves in the same order.

But it is not legitimate to assume that each leaf on the plant begins its history with the same constitution and repeats in its turn the same succession of physiological changes, at the same rate, as every other; if this were true, then and then only would the successive leaves at any time represent stages in the development of a single leaf. Underlying work of the kind under discussion, therefore, is the assumption that the successive leaves produced by a plant, if taken at corresponding stages, form a uniform population whose distribution is determined entirely by chance causes, and that there are no general drifts dependent on the time during the life history of the plant at which the individual organs are produced. That such an assumption has been made appears to be often entirely unrecognized, but it is nevertheless totally unwarranted. In fact, so complex

appears to be the relationship between the age changes of many characteristics of the successive leaves that it frequently becomes impossible to define 'corresponding stages' among the various members with any approach to accuracy.

The successive leaves on a shoot usually attain very different sizes, shapes, and colour; and an indication that more fundamental characteristics may be quite dissimilar is given by the fact that the foliage leaf series is not continued indefinitely, but after undergoing readily observed transitions in the above characteristics may be directly succeeded by an apparently new kind of member. Very probably underlying the sudden morphological change there has been a more gradual physiological transition. The growing point in fact produces a succession of foliar primordia which from their inception may be very different in both constitution and potentialities, for the meristem itself has a well-defined life history, and in the production of its members reflects the general age of the shoot, or plant, including changes in the supply of nutrients and food materials.

In the leaves on a plant, therefore, one is concerned with two age drifts, the age of the individual leaves and that of the shoot; and these are in a sense opposed, leaves which are of the most advanced age having been formed by the shoot while the latter was in a comparatively young stage, and vice versa.

Characteristics of the successive leaves of barley, and their changes with age, have been extensively studied in this Institute. The results are striking in that in almost every observed respect the successive leaves are so unlike as to invalidate any attempt to deduce the age sequence of a single leaf from the particular values observed in successive members at any one time. Some of these results have already been published (2 and 6), e.g. the ratios of dry weight to leaf area and of water to leaf area, water content, potassium content, rate of respiration and rate of carbon assimilation of the successive leaves immediately after complete expansion. Many more data as yet unpublished are available for carbohydrate and nitrogen fractions. From these it is clear that it is impossible to predict what course any particular characteristic will take throughout the leaf series. The ratios of dry weight to leaf area and of protein nitrogen to total nitrogen, for example, do not show any very large variation in successive leaves of well nourished plants, but under certain conditions may differ more widely, e.g. when potassium is lacking. Nitrate nitrogen, on the other hand, rises to a maximum in some particular leaf and declines rapidly in the later ones, while percentage dry matter falls to a minimum and afterwards rises. In other cases the relationship may be more complicated—for example, in total nitrogen expressed as a percentage of dry weight the value is maximal at leaf 2 or 3 and minimal at 8 or 9, showing a real rise again in the last leaves produced.

More important still for the present purpose is that the variation from leaf to leaf may be much greater than the change with age within any particular leaf; and that in fact not only is there this primary difference between the individual leaves, but age does not necessarily have the same effect on one leaf as on another, and a statistically significant interaction may be obtained between leaf number and leaf age. In illustration of this last statement the variation found in total nitrogen as a percentage of dry weight in the leaves of barley may be given. After complete expansion, in the first leaf, the nitrogen content fell very slowly from 4.92 per cent. to 3.54 per cent., the last observation taken before death. In leaves later than the first the life history is shorter and the rate of fall with age is much more rapid; the last leaves, however, which again have longer histories and presumably intercept the upward translocation of nitrogen to the developing ear, continue to accumulate nitrogen for a considerable time after complete expansion. It is also interesting to note that the lowest observed values in the first few leaves are actually higher than the highest in the last four leaves produced.

As an example of the unwarranted conclusions which have been drawn from data in which the leaves present on a shoot at one time have been used as an age series may be cited some respiration results of Hover and Gustafson (3). Working with maize, sorghum, oat, wheat, and sunflower, they claim to have shown that in every case except the last the respiration rate of leaves at first decreases with age to a minimum, but subsequently, 'past about middle age', increases again. Now it is known (2, 6) that in barley the successive young leaves do not have the same respiration rate, but that those first formed have a higher rate than the later ones. Taking as a first approximation that in general the earlier a leaf is produced the higher is its respiration rate, and that during the life history of each leaf its respiration rate falls more or less proportionately to its initial value, the results of Hover and Gustafson can readily be explained. Such a system may be represented by the scheme shown in Fig. 1, where curve 1 represents the respiration-time relation of the last-formed leaf, and curve 8 that of the first-formed (oldest) leaf. This is the notation used by Hover and Gustafson. These leaves having in fact been produced at more or less equal intervals of time, the respiration-leaf number curve at a time in which these eight leaves are all simultaneously living on the shoot may be directly obtained by taking equally spaced intercepts on the curves along the time axis. This curve is represented by points A1-A8, where A1, A2, &c., represent the respiration rates attained at this time by successive leaves. The usual relationship found by these workers is precisely of this type, and there is clearly no justification for assuming, as they do, that it represents in any way the respiration-time relation of single leaves, or that this curve is identical with those from which it is obtained, namely 1-8.

In reality there is no doubt that the facts are more complicated, since the curves 1-8 have been assumed to be exactly similar, whereas they are known to differ somewhat among themselves. That the explanation given,

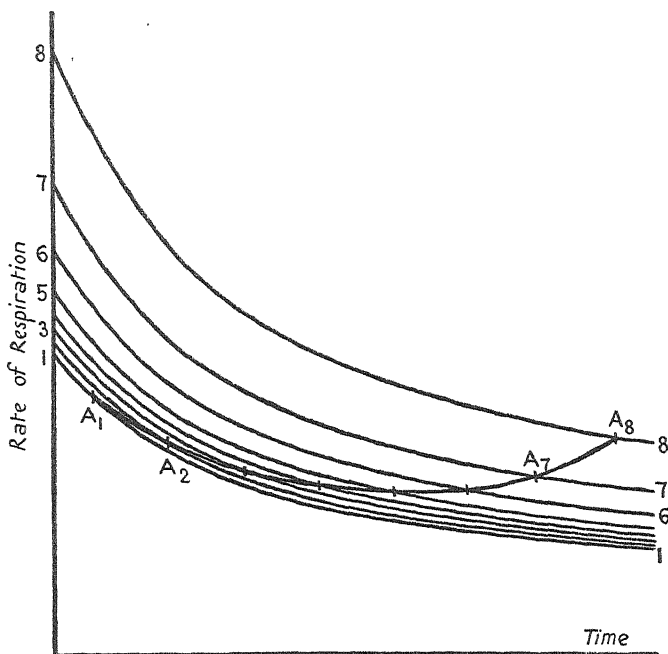


FIG. 1. General scheme for the respiration rate-time relation of the successive leaves on a shoot, and for the respiration rate-leaf number relation at any given time. For explanation see text.

however, is a first approximation to the truth is evident from one of the diagrams given by Hover and Gustafson, and reproduced with additions in Fig. 2. In this, curves are presented of the rates of respiration of all the leaves present on maize plants at different stages of growth: 3-, 4-, and 10-leaved stages. The authors state that 'since there were variations in the duration of the experiments and also in the temperature conditions, the rates of respiration in one experiment cannot, *except in a general way*, be compared with the rates in another'. The italics are not in the original. The stated differences in the duration of the experiments are certainly insufficient to account for the very different levels of curves A, B, and C, and it is most unlikely that seasonal variation or the temperatures within the laboratory could do so, though these explanations are the only ones offered to account for the large divergences.

If these curves are compared, 'in a general way', it is at once clear that the third leaf in A becomes the fourth in B, and the tenth in C, or possibly some leaf which has already withered; this, however, does not



affect the general argument. When the points representing the respiration rates of this leaf at these three stages are joined a continuously falling curve is obtained. By joining other corresponding points in their diagram a set

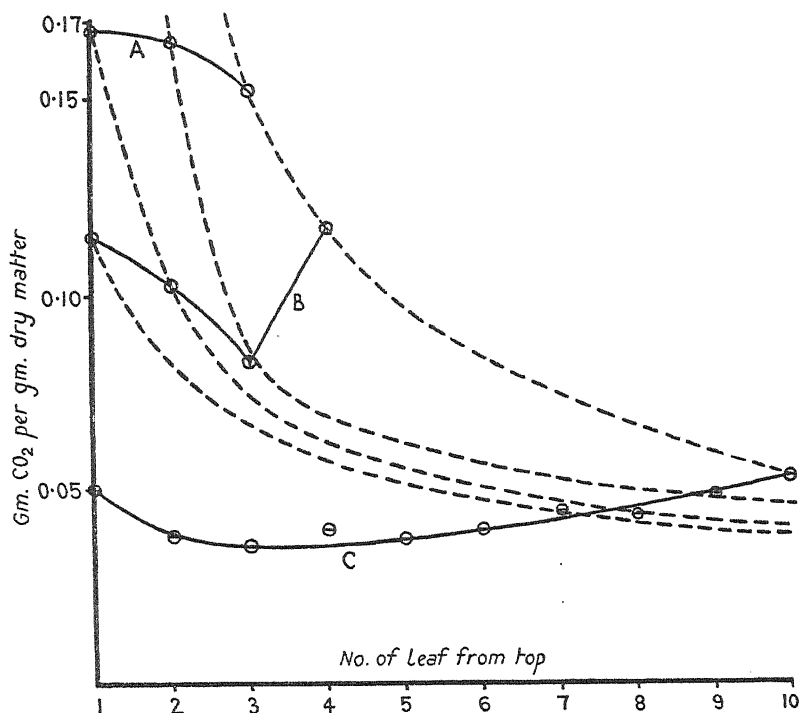


FIG. 2. Respiration rates of the individual leaves present on maize plants at different stages of growth: A 3-, B 4-, and C 10-leaved stages. After Hover and Gustafson.

of four curves similar in spacing to those of Fig. 1 is obtained, three being determined by three points each, and the fourth by two. From this diagram then it is evident that a young leaf when the plant is in a 3-leaved stage has a higher respiration rate than one of a corresponding age when the 4-leaved stage is reached, and this again is much higher than that of a young leaf from an older plant—a result that entirely confirms those obtained for barley by the author.

More recently Pope (5) performed precisely similar respiration experiments, using the leaves present at one time on the tillers of barley, and concluded that 'the rate of respiration was high in young barley leaves and decreased in proportion to the rapidity with which the leaves matured. Catalase activity was low in young barley leaves and increased to a maximum at about the time of leaf maturity, after which it decreased'. He therefore found a negative correlation 'between respiration rate and

catalase activity in homologous younger leaves. In older leaves relationships were indefinite.' The same confusion between leaf age and leaf number thus exists, and his results are accordingly difficult to interpret with precision. The difference between his respiration rate results and those of Hover and Gustafson is doubtless due to the fact that Pope used only the later leaves of the series produced on a tiller, and not the earlier. It has been shown (2, 6) that the differences in respiration rate between the successive leaves of barley at corresponding leaf ages are greatest among the earlier ones, later ones showing comparatively slight differences. If therefore these later leaves alone are tested simultaneously the result to be expected is a continuously falling curve similar to the first half of A I-A 8 in Fig. 1, and that is precisely the form found by Pope. If on the other hand the series is tested at a still greater age, so that the last leaf produced on the shoot is already of considerable age, it is evident that a falling curve need no longer be obtained, a rising one being at least as probable. This is the condition in the oat and wheat experiments of Hover and Gustafson, in which an increasing rate with 'age' was generally observed.

Pope's catalase results again must be compared with the curve A I-A 8 in Fig. 1, and do not at all necessarily reflect the effect of leaf age alone on its activity. It is, moreover, entirely false to correlate catalase activity with respiration rate in his leaf series unless it is reasonably certain that other factors affecting respiration rate are nearly constant. In his 'homologous younger leaves' it is very probable, from similar observations made at this Institute, that the successively younger leaves had rapidly increasing sugar contents, besides possibly increasing nitrogen and protein contents; the negative correlation he observed in his youngest leaves may very likely be caused entirely by differences in concentration of sugar or amount of protoplasm, and therefore these particular data do not justify his final conclusion on the relationship between catalase activity and respiration rate: 'it is highly probable that any correlation is fortuitous'.

One final example from the literature may be given. Pearsall (4) in 1931 determined various nitrogen fractions in the protein of six selected leaves, covering a range of 26 leaves, from shoots of *Beta vulgaris* v. *cicla*, assuming these to form a simple age series. This series he divided into two portions—leaves in the 'growth' stage and those in the 'photosynthetic' stage. He found that the older the leaf the lower was the ratio of basic-N to non-basic-N, i.e. roughly speaking, diamino-N to mono-amino-N; this value fell rapidly at first but became nearly constant among the older leaves. From a comparison of this change with corresponding changes in leaf length and leaf weight he concludes 'it is clear that growth metabolism incorporates a far greater proportion of basic-N into the proteins than does the manufacture of photosynthetic protein'. Such a conclusion, if true, is important, and for this reason the experimental evidence on which

it rests should be above criticism. Since the result depends on the supposedly much higher correlation with leaf length than with leaf weight, in the light of the previous discussion it is evident that a complex assumption is involved, i.e. change during growth in the ratio of basic-N to non-basic-N, as compared with change in (a) length and (b) weight, is the same from leaf to leaf. The simplest conditions under which this can be fulfilled are that (1) at corresponding ages in the successive leaves the ratio value is equal, and similarly (2) at corresponding ages length and weight are equal in all members, and therefore the ratio of length to weight is constant at a given age, and all leaves attain the same final length and maximum weight.

Admittedly, on *a priori* grounds, the errors due to these assumptions may not be so great as those in the previous papers discussed, for the following reasons: Chibnall (1), using all the leaves present on the plant, found the various fractions in the proteins of runner-bean leaves to be remarkably uniform from a 6-leaved stage to a 372-leaved stage; and in barley supplied with ample nutrients (2, 6) the ratio of leaf area to dry weight is rather more constant in successive leaves than are most leaf characteristics. But comparatively slight errors due to these causes would be sufficient to alter the general trend of the correlations Pearsall found; it is unfortunate that so important a conclusion should rest on such doubtful premises.

*Conclusion.* Only in very exceptional cases is it permissible to use the leaves present on a shoot at one time as representative of a simple age series from which the history of single leaves at successive stages may be deduced. Included in the differences observed between the members are differences due to the fact that, even at comparable ages, the successive leaves constitute a series of inherently different physiological structures. To determine the effect of age on a particular leaf the corresponding leaves at various ages on replicate plants must be used, even though this involves larger errors of sampling. Further, it must be recognized that the age effect need not be uniform from leaf to leaf. Finally, it is impossible to separate effects which may be possibly ascribed to age as such from those due to change in conditions of nutrition, &c. As a leaf ages it becomes further removed from the growing point and passes successively from the position of the topmost leaf on the shoot to that of the lowest living leaf, a change which in itself must have a far-reaching effect.

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# Anomalous Secondary Thickening in Compositae.

BY

R. S. ADAMSON.

With seven Figures in the Text.

IN the course of some investigations on wood structure examples were found of anomalous secondary growth in certain of the shrubby members of the Compositae in the South-western Cape region of South Africa. This anomaly is a type that does not seem to be recorded in this family. Deviations from the normal method of secondary thickening have been recorded in several members of the Compositae (5), but these are either in climbing plants or tuberous stems.

The cases described at present are small shrubs with hard woody stems and without obvious external peculiarity. The anomalous growth has been found in the following genera: *Lachnospermum*, *Elytropappus*, *Disparago*, *Stoebe*, *Perotriche*, and *Phaenocoma*. All these plants are woody, with very hard stems characterized by possessing very thin bark. The following measurements will illustrate the point. In making them, the cambium line along which the bark peels off was taken as the limit of 'bark'.

Species.	Total diameter. mm.	Thickness of bark. mm.
<i>Metalasia muricata</i>	11.2	0.2
<i>Elytropappus rhinocerotis</i>	10.4	0.3
<i>Phaenocoma prolifera</i>	6.2	0.2

In the stems of these plants secondary growth commences at an early period. The secondary tissues are formed by an extrafascicular pericyclic cambium which adds to the tissue by divisions cut off on the inside. The new tissue consists of strands of xylem and phloem, arranged radially to one another, embedded in ground-tissue that is usually lignified. The type of secondary is exactly like that which is found in *Arthrocnemum* or *Bougainvillea* and some others of the Centrospermae (cf. Pfeiffer, where full references are given). The cambium layer that forms is persistent throughout. There is no development of successive cambia as in many Amarantaceae (5, 6).

*Primary stem.* The primary stem in all the plants is of small diameter with the bundles arranged round a central pith that becomes lignified. The primary bundles are without cambium (Fig. 1). At the time differentiation is completed all the cells between the xylem and phloem become lignified. Lignification also occurs throughout the phloem with the exception of the sieve-tubes and their companion cells. Fibres are found immediately outside the phloem.

External to the bundles is a single layer of parenchyma cells forming a pericycle. The innermost layer of the cortical cells are enlarged and appear empty. The walls of this layer become suberized at an early stage, before any secondary growth has commenced. The cortex beyond is very thin, and becomes dried up soon after the suberization is completed.

All the plants have small, more or less ericoid leaves, each of which has a single leaf trace. The leaves persist on the stems some time after secondary growth has commenced. The departing trace is accompanied by a prominent leaf-gap.

*Cambium.* The cambium arises from the layer immediately internal to the large suberized cells and just external to the primary bundles. At first the cambium is a single layer, the division of which adds to the tissue inside. At first no divisions occur to the outside; the layer of suberized cells persists and its cells become tangentially stretched. Later, with increasing diameter, this layer is ruptured and new cork cells are added by external division of the cambium zone which has become broader; 3-5 cells in *Metalasia*, but wider still in *Phaenocoma* and *Elytropappus*. In old stems of *Phaenocoma* and *Metalasia cephalotes* the cork forms a thick covering, but quite a thin one in the others. The primary cortex which persists for some time is finally cast off when cork formation commences.

*Secondary tissues.* With the exception of the sieve-tubes and their companion cells all the cells formed from the cambium become lignified. The secondary tissues are in immediate juxtaposition to the primary bundles; there is no gap between them. In the secondary tissues considerable displacement of cells occurs as the result of enlargement of vessels, &c. The phloem strands in the secondary tissues are easily recognized owing to the presence of very thick-walled fibres around the sieve-tubes, and especially to the outside. This development of fibres in the phloem occurs in all except *Metalasia*, where few are formed. The actual form of the secondary tissues shows small differences in the different genera.

In *Metalasia* the strands of xylem and phloem are quite small, the phloem especially consisting of 3-4 sieve-tubes only. The secondary tissue is traversed by broad rays of lignified cells. In old stems distinct concentric rings are formed as the result of differences in the size and thickening of the cells of the ground tissue (Fig. 2).

*Phaenocoma* is similar to *Metalasia*, but the strands of xylem and phloem are larger, rays are not distinct, and there is no formation of rings.

*Elytropappus rhinocerotis* has distinct bundle-like strands scattered in

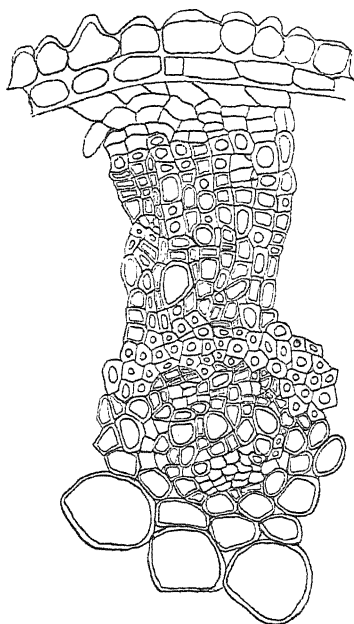


FIG. 1.

FIG. 1. *Stoebe cinerea*. Transverse section of stem showing primary bundle and beginnings of secondary tissues.  $\times 310$ .

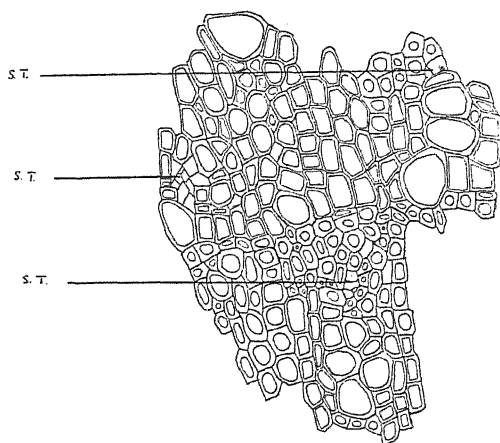


FIG. 2

FIG. 2. *Metalasia muricata*. Part of transverse section of old stem with ring formation. S.T., groups of sieve-tubes.  $\times 200$ .

lignified ground tissue with no definite rays and no rings. The xylem strands contain 1-2 rows of 5-6 radially placed vessels; the phloem has a few sieve-tubes scattered among very thick-walled fibres. In *E. microphyllus* the xylem and phloem are in strands which are widened tangentially, giving the whole mass the appearance of concentric zonation.

Four species of *Stoebe* have been examined, in all of which this tangential extension of the strands is more developed. The xylem and phloem are flattened strands separated from one another by lignified ground tissue. This gives a definite concentric appearance which is emphasized by the small very thick-walled fibres external to the phloem.

*Perostriche* is anatomically indistinguishable from *Stoebe*.

Two species of *Disparago* have been examined. These are similar to *Stoebe*, but the flattened strands are only connected by the fibres; the xylems and phloems form a series of small parallel strands separate from one another.

*Lachnospermum* has a less regular structure. The ground-tissue is almost confined to rays which are six or more cells across. Between the rays the tissue is made up of xylem with strands of phloem. The phloem

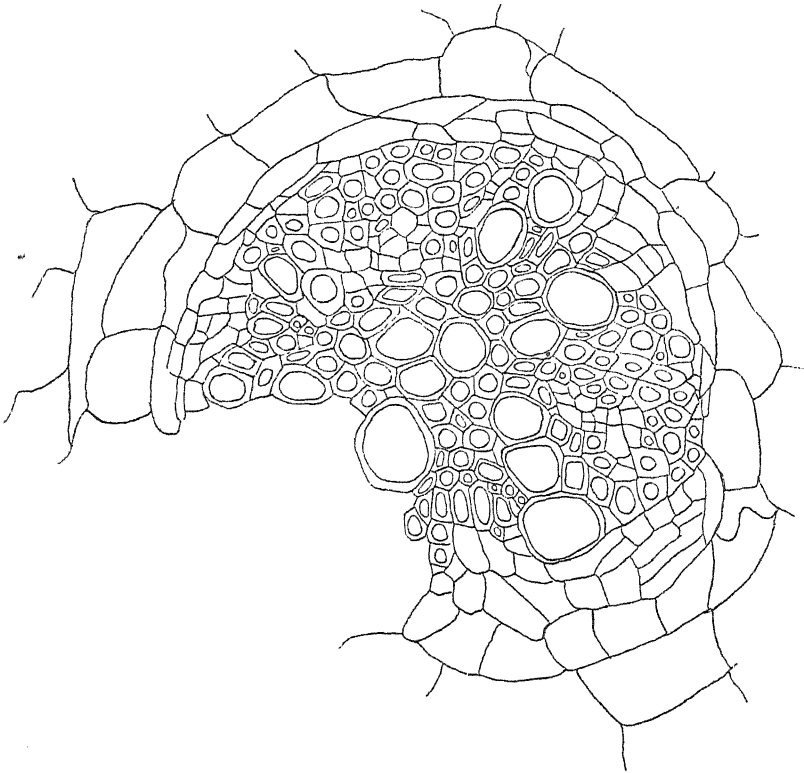


FIG. 3. *Metalasia muricata*. Transverse section of centre of 3-arch root just previous to secondary growth.  $\times 250$ .

strands consist of a small group of sieve-tubes ensheathed by a band of small hard fibres.

*Root.* The roots are hard and wiry, often extensive but not very freely branched.

While the course of secondary growth in the stems in all the genera is essentially uniform, differences occur in the roots.

In all the plants secondary growth commences early. In *Metalasia muricata* roots with a diameter of 0.5 mm. had the beginning of secondary tissue. A root from the same plant in which primary differentiation was just completed measured 0.44 mm.

In *Elytropappus rhinocerotis* roots with primary differentiation just completed measured 0.41 mm. and secondary increases commenced without increase in diameter. The primary roots are usually 3- or 4-arch, less



commonly 2- or 5-arch. As in the stems, all the cells except sieve-tubes, of the phloem, both primary and secondary, become lignified.

*Metalsia*. The primary 3- or 4-arch root does not form a cambium

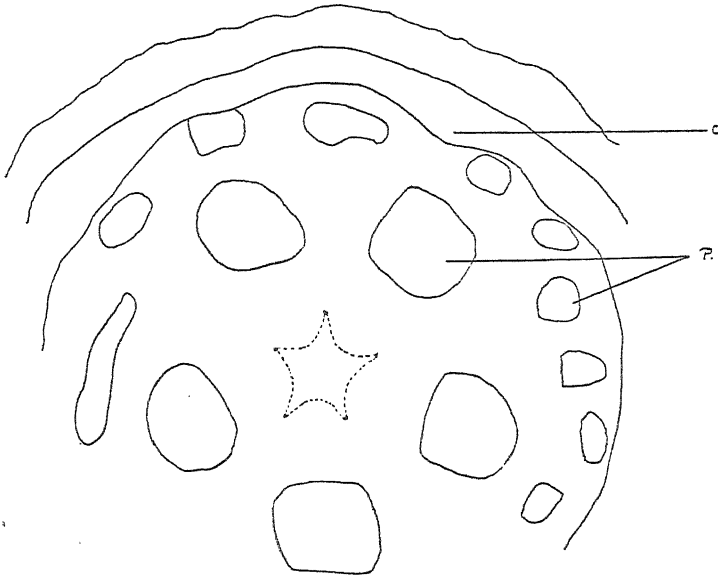


FIG. 4. *Phaenocoma prolifera*. Transverse section of root. P., phloem; C., cambium.  $\times 50$ .

between the xylem and phloem. The ground tissue-cells in this position all become lignified and are mostly transformed into xylem elements (Fig. 3). A cambium arises from the pericycle which behaves and develops exactly as does the cambium in the stem and the secondary tissues formed are identical with those in the stem. There is no difference in structure between a stem and a root in *Metalsia* except for the arrangement of the primary tissues.

*Phaenocoma*. A different structure is present in this plant. The roots are thicker and generally 4- or 5-arch, with a distinct lignified pith. After the completion of the primary tissues a cambium is formed in the normal position and carries out divisions in the ordinary way, though forming much less phloem than xylem, and forming phloem only next to the primary phloem strands. Cambial growth continues till the xylem forms a fluted cylinder with the phloem occupying the grooves. The whole is cylindrical and of the so-called 'Bignonia' type. The diameter of this fluted cylinder may be as much as 1.66 mm., though usually less. Subsequently the cambium internal to the phloem ceases division and a cambium is formed external to the phloem strands. This external cambium, which is a continuous ring, forms additions to the inside exactly as happens in

the stem (Fig. 4). In old roots the cambium forms a broad zone 8-12 cells in thickness and produces cork externally. When this external cambium is formed the original cambium internal to the phloem remains

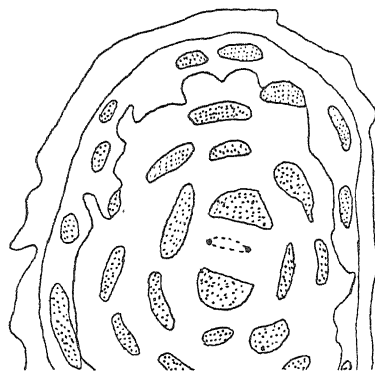


FIG. 5. *Elytropappus rhinocerotis*. Transverse section of old root showing distribution of phloems (dotted) and 'phloem islands'.  $\times 35$ .

unaltered as a line of thin-walled cells surrounded and enclosed by cells with lignified walls. In the subsequently formed phloem strands there are no such thin-walled cells present, though the fibres show no change in diameter or sectional outline from the cambium cell from which they arose.

*Elytropappus*. The young roots are very slender and are 3- or 4-, less often 5-arch. Secondary growth commences normally just as in *Phaenocoma* and forms a fluted cylinder of the 'Bignonia' type. This cylinder is, however, quite small; the largest seen was 0.5 mm. in diameter, generally it is much less. Subsequent growth produces rather irregular concentric zones with strands of xylem and of phloem (Fig. 5). The phloems have fibres externally. Internal to each phloem is a line of cambium-like cells, though this is often incomplete. In old roots all the cells may become lignified.

The formation of these later secondary tissues is not the same as in *Phaenocoma*. As in that genus, an external cambium forms round the original fluted cylinder and forms a zone 8-12 cells in thickness. From this cambium xylem and ground-tissue are differentiated by internal divisions, but differentiation of phloem commences in the middle region of the cambium and additions are made centripetally by the cambium cells on the inside. The phloems thus formed appear at first as islands in the cambium zone. In the growth of the phloem all the cambium cells may be transformed to permanent tissue, or a single line may remain, which ceases division. As xylem is forming on either side of these phloem islands, the outer margin of the woody tissue is fluted. The cambial cells lateral to the phloem islands become changed to permanent tissue.

Subsequent growth, after the formation of a phloem island, is carried out by the outer cells of the cambium zone (Figs. 6 and 7).

*Stoebe*. The plan of construction of the root is in all essentials like that in *Elytropappus*. In *Stoebe capitata* the cambium cells internal to the phloem strands, which are tangentially extended, become wholly transformed

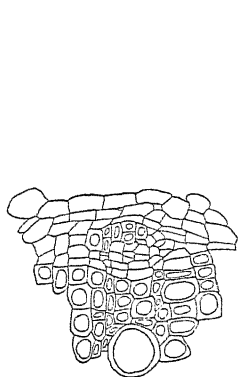


FIG. 6.

FIG. 6. *Elytropappus rhinocerotis*. Part of section showing early stage in formation of a 'phloem island'.  $\times 300$ .

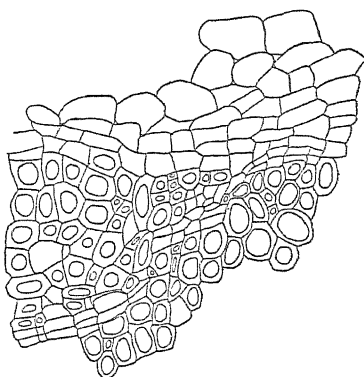


FIG. 7.

Fig. 7. *Elytropappus rhinocerotis*. Phloem island completed.  $\times 300$ .

into permanent tissues; whereas in *S. cinerea* and other species a distinct line of cells with thin walls persists just internal to the phloem even in old roots.

*Disparago* has exactly the same arrangement, with persistent thin-walled cells internal to the phloem.

### DISCUSSION.

The genera in which this anomalous secondary growth has been observed are allied to one another. They all belong to the tribe Inuleae, but represent two of the sub-tribes: *Phaenocoma* is placed in the Gnaphalinae, while the other genera belong to the Relhaniinae (cf. Hoffmann).

In attempts to discover the extent to which anomalous growth occurs in the family, the following allied genera have been examined in addition to those described:

- Gnaphalinae. *Gnaphalium*, *Helichrysum*, *Helipterum*, *Petalacte*,  
*Anaxeton*, *Leontonyx*.
- Relhaniinae. *Bryomorphe*, *Relhania*, *Nestlera*.
- Athrixiinae. *Athrixia*, *Leyssera*.
- Filaginae. *Ifloga*.

In all these genera secondary growth is normal in its formation. The plants do, however, show some features in common with those described. In both stem and root the phloem becomes lignified except for the sieve-tubes and companion cells. In both root and stem secondary phloem is added only at the positions of the original phloems and the xylem may

become slightly fluted. For example, in *Petalacte*, where the primary root is diarch, the secondary phloem strands together occupy less than a quarter of the circumference of a four-year-old root.

Of these other genera examined, *Nestlera*, *Relhania*, and *Ifloga* alone form woody bushes comparable to those of the forms with anomalous growth. The others are more or less herbaceous and very often without persistent woody aerial stems.

Anatomically, *Athrixia* seems to represent an extreme state in the tendency towards the herbaceous habit. The underground part of the stem has normal continued secondary growth, but the flowering shoot has limited growth. Secondary tissues are only added in the original bundles, no interfascicular cambium being formed. The cells of the rays between the bundles become completely lignified.

In the roots especially the similarity in structure is very striking. The early stages in the roots of *Elytropappus* or *Stoebe* are almost identical with corresponding sizes in *Helichrysum* or *Petalacte*, which have the limited strands of secondary phloem.

Apart from the anomalous thickening these plants are anatomically closely allied to their systematic allies, and it would seem a possible view that the anomaly has arisen as a means of attaining the shrub life-form by a plant that was wholly or partially herbaceous. These plants have no normal cambium in the stem and develop the anomalous one from the pericycle.

These plants might be quoted as examples in support of what Mrs. Arber has termed the 'Law of Irreversibility', that an organ or structure lost cannot be recreated but may be functionally replaced by some new development. Against this, however, may be placed the curious root behaviour in all except *Metalasia*. The root is generally looked upon as a conservative organ, and the persistence, even partially, as in these plants, of normal secondary growth might be taken to represent an ancestral character. But a partial loss of cambial activity is not readily explained by the 'Law of Irreversibility'.

To return to the systematic arrangement, the genera *Elytropappus*, *Stoebe*, *Perotriche*, and *Disparago* are exceedingly closely allied to one another, so closely, indeed, that there may be some doubts as to the validity of maintaining all the genera. *Metalasia* and *Lachnospermum* are also very nearly related, and these, with the first group, form a compact division in the Relhaniinae, easily separated from *Relhania*, *Nestlera*, and others. Along with the first group are included three other genera, *Bryomorphe*, *Amphiglossa*, and *Syncephalum*. The two last have not been examined, but the first which has normal structure is a cushion plant with short, closely crowded shoots, which has achieved the herbaceous habit.

*Phaenocoma*, which has nearly the same habit and anatomy as these

others, is separated by its floral characters and associated with *Helichrysum*, to which indeed it was united by Baillon. It is especially associated with *Petalacte* and *Anaxeton* in having the central flowers sterile and the outer female. Thus if the grouping represents real affinities it would appear that this form of anomalous thickening may have arisen more than once from nearly allied ancestors.

The general hypothesis that this anomalous thickening is a character acquired by the plants which have developed from more or less herbaceous ancestors is supported by the views expressed by Small in regard to the evolution of the family. He states that *Gnaphalium* arose early [in the Tertiary epoch and represents the earliest of the sub-tribes. *Helichrysum* was differentiated in the early Miocene and the Relhaniinae about the end of that period. In his schematic diagram Small indicates (New Phyt., xviii, fig. 79) a different origin for *Relhania* on the one hand and *Metalasia* on the other, the latter being derived from *Helichrysum*.

But while these views seem to gain support from the anatomical data that have been put forward it is not proposed to follow them out at all. The plants with anomalous growth form a group of forms closely related in many features, and one which has achieved a distinct element of success. Both *Metalasia* and *Elytropappus* contain species which appear to have a progressive and expanding area of distribution.

#### SUMMARY.

Anomalous secondary growth occurs in seven genera of Compositae belonging to the Inuleae in the sub-tribes Relhaniinae and Gnaphalinae.

In the stem the primary bundles are without cambium. A pericyclic cambium arises which adds internally strands of xylem and phloem with ground-tissue.

In the root a pericyclic cambium which forms tissues just as in the stem occurs in *Metalasia*. In the others the root at first grows normally and reaches a 'Bignonia' type. Subsequently an external cambium, with growth as in the stem, is formed in *Phaenocoma*. In *Elytropappus*, *Stoebe*, and *Disparago* the cambium zone formed round the original cylinder forms xylem inside and phloem as 'islands' in the cambium zone. The phloem islands continue differentiating centripetally. Later growth continues external to them.

The phloem in both stems and roots is lignified. This character is common to all members of the sub-tribes.

It is suggested that the anomalous secondary growth arose in plants which were herbaceous in habit.

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# Interaction of Factors in the Growth of Lemna.

## V. Some Preliminary Observations upon the Interaction of Temperature and Light on the Growth of Lemna.

BY

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With an Appendix by ERIC ASHBY.

With four Figures in the Text.

IN the following experiments colonies of *Lemna minor* were grown at light intensities from 80 to 1,400 foot candles, and at temperatures from 15° to 35° C. The apparatus used was that described by Ashby (1), in which colonies of *Lemna* can be grown at four different light intensities in one and the same nutrient solution, the temperature of which can be controlled to within 0.5° C.

The measures of growth taken were frond number, area, and dry weight, and the technique employed was that already in use in this laboratory. In addition to these observations the respiration rate and chlorophyll content of fronds were followed throughout certain experiments.

The aim of the experiments was to construct a 'surface' on which should be recorded the relative growth rate of *Lemna* at any combination of light and temperature. In order to provide points for this surface, experiments were carried out under the following conditions :

Expt. 1.	15° C.	light intensities :	350, 750, 1,000, 1,400, f. c.
" 2.	22.5° C.	light intensities :	350, 750, 1,000, 1,400, f. c.
" 3.	25° C.	" "	350, 750, 1,000, 1,400, f. c.
" 4.	30° C.	" "	350, 750, 1,000, 1,400, f. c.
" 5.	35° C.	" "	350, 750, 1,000, 1,400, f. c.
" 6.	25° C.	" "	80, 120, 180, f. c.
" 7.	22.5° C.	" "	100, 140, 210, 250, f. c.

For two reasons the results obtained from these experiments must be regarded as tentative. In the first place, although every experiment was begun with a homogeneous population of fronds, the initial populations in the several experiments differed as to frond weight, frond area, and chlorophyll content. While it is possible, therefore, to compare the frond area, frond weight, &c., at different light intensities in the *same* experiment,

it is not legitimate to compare these values *between* experiments. In the second place it will be observed that there is only one value of the relative growth rate for each combination of light and temperature. The question arises as to whether an experiment at 15° in January can be compared with an experiment at 30° in March. There is no accurate information as to the reliability to be placed on a *single* experiment at any one temperature, and this source of error has accordingly been neglected in discussing the following experiments.<sup>1</sup> An improvement in the technique of earlier work was made by continuing certain experiments for seventeen days. This procedure reduced considerably the standard error of the relative growth rates.

### SUMMARY OF RESULTS.

1. *Fronde number.* The increase in frond number in Experiments 1 to 4 was exponential at all light intensities. The logarithms of frond number plotted against time fell conformably along straight lines. If regression equations are fitted to these straight lines, the regression co-efficients, which are the slopes of the lines, are a measure of the relative growth rates. The results of the experiments may conveniently be summarized by drawing up a table of the regression co-efficients under different conditions of light and temperature. The relative growth rates, under all conditions at which growth is exponential, can be gathered from the regression co-efficients set out in Table I.

TABLE I.

*Regression Co-efficients at Different Combinations of Light and Temperature.*

Light intensity :—		350	750	1,000	1,400
Temperature.					
15°		0.03204	0.04474	0.04169	0.03913
	S.E.	0.00224	0.00130	0.00156	0.00748
22.5°		0.08699	0.09314	0.10096	0.07918
	S.E.	0.00064	0.00099	0.00089	0.00117
25°		0.09402	0.11350	0.12011	0.12533
	S.E.	0.00111	0.00043	0.00048	0.00742
30°		0.08905	0.12483	0.13114	0.12117
	S.E.	0.00216	0.00109	0.00124	0.00156

It is clear that the growth of colonies of *Lemna* is exponential between 15° and 30°, and between light intensities of 350 and 1,400 foot candles. In all experiments performed *outside* this range of light or temperature the

<sup>1</sup> In an attempt to estimate the reliability of a single experiment at any one temperature, a duplicate experiment was carried out at 15° C. three months after Experiment 1. The relative growth rates did not differ significantly in the two experiments, but the standard errors in the second experiment were so large that the result cannot be considered conclusive.



growth was not exponential, and the relative growth rate fell with time. The range of temperatures and light intensities in Table I may therefore be taken as a rough indication of the range of adaptation of *Lemna*,

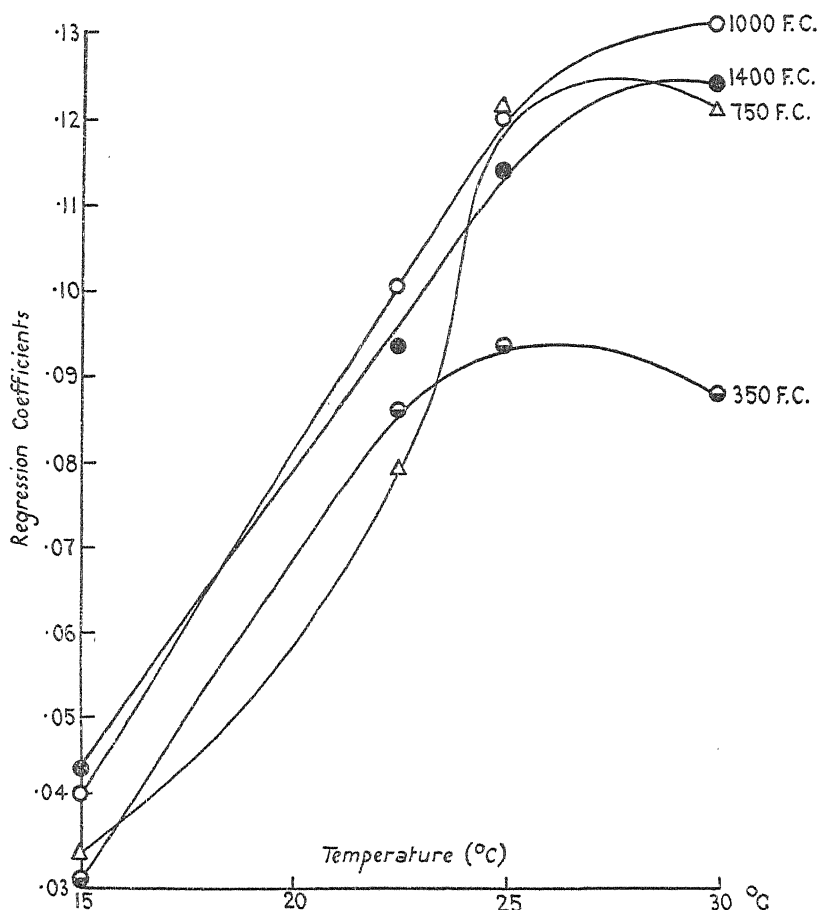


FIG. 1. Regression coefficients of growth curves at different light intensities plotted against temperature. For explanation of regression coefficients see text, p. 516.

except that there is no information as to the behaviour of this strain at intensities above 1,400 foot candles.

From Table I the following conclusions can be drawn. Between 15° and 22.5° the rate of increase of relative growth rate with temperature is almost independent of the light intensity. The values of the regression co-efficients at each light intensity are plotted against temperature in Fig. 1, and from the average slopes of the curves in Fig. 1 the temperature co-efficient of growth can be calculated. The value of  $Q_{10}$  is 2.854. The optimum temperature for growth is in the region of 30° C.

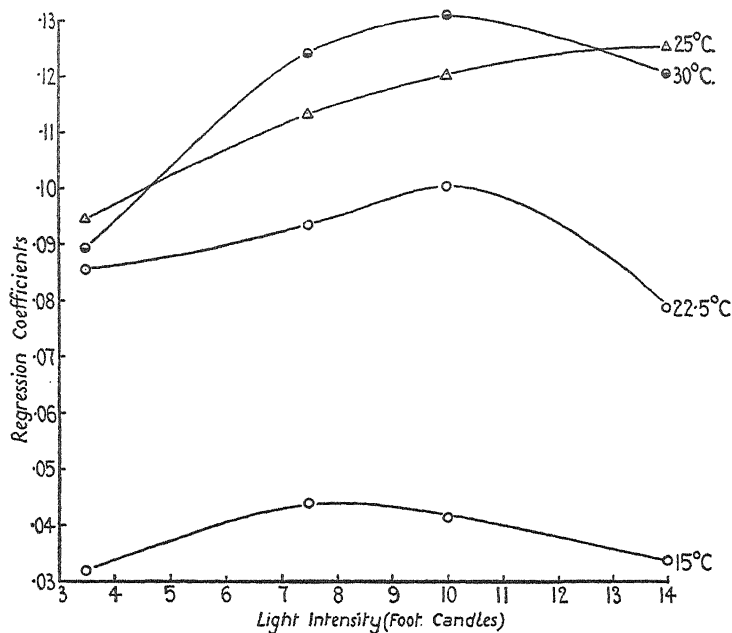


FIG. 2. Regression coefficients of growth curves at different temperatures plotted against light intensities.

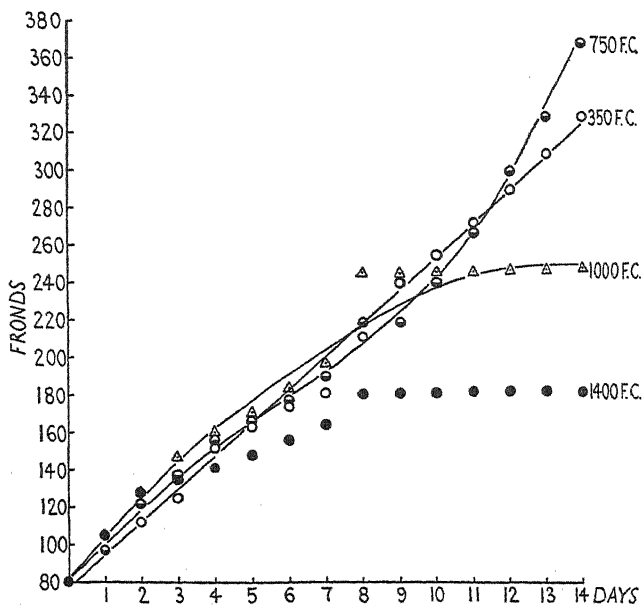


FIG. 3. Frond numbers plotted against time. Colonies grown at 35° C. and four different light intensities.

The relative growth rate increases with light intensity to an optimum between 1,000 foot candles and 1,400 foot candles. At  $22.5^{\circ}$  and at  $30^{\circ}$  the relative growth rate is significantly lower at 1,400 than at 1,000 foot

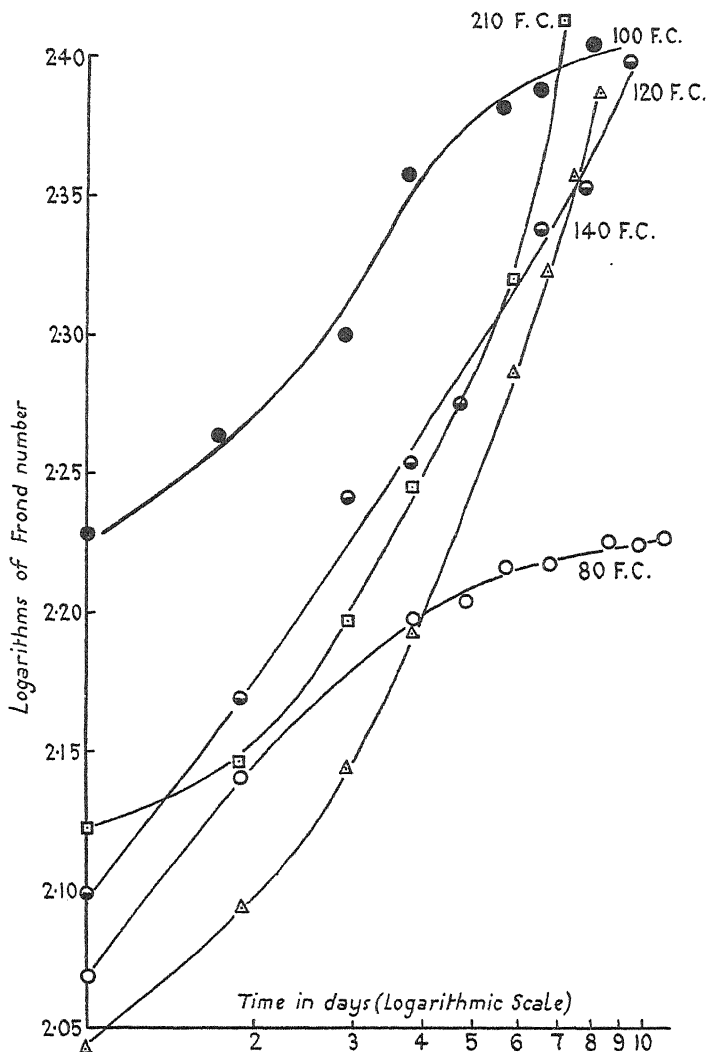


FIG. 4. Experiments at low light intensities. Logarithms of frond number plotted against logarithms of time. Temperatures  $22.5^{\circ}$  and  $25^{\circ}$  C.

candles. At  $15^{\circ}$  and at  $25^{\circ}$  there is no significant difference in the relative growth rates at these two light intensities. This low light optimum for the growth of *Lemna* has already been reported by Ashby (2) for another strain. The optimum light intensity may differ for different temperatures,

but the data to hand are insufficient to provide conclusive evidence on this point. In Fig. 2 are plotted the values for the regression coefficients at different temperatures against the light intensity. A combination of Figs. 1 and 2 give the light-temperature surface, and so far as the data are valid, they indicate that the optimum conditions for the growth of this strain of *Lemna* occur at 1,000 foot candles and 30° C.

In Experiment 5, at 35° C., the relative growth rate fell with time at all light intensities. The curves of frond number are plotted against time in Fig. 3, and it will be observed that even at this supra-optimal temperature, light still has a significant influence on the growth of the colonies. At 350 and 750 foot candles the growth is not far short of exponential: at the higher light intensities the relation between frond number and time is more nearly parabolic.

Experiments 6 and 7 were carried out in order to study the effect of low light intensities. Under all conditions in these experiments the relative growth rate falls rapidly in time. When the logarithms of frond number are plotted against the logarithms of time, a series of curves is obtained, some concave to the time axis and some convex (Fig. 4). These resemble the family of curves Gregory (4) obtained when cucumbers were grown at low light intensities. It is of interest that, although these light intensities are above the compensation point (which appears to be a little below 80 foot candles), they will not support the unrestricted growth of *Lemna*.

2. *Frond weight and frond area.* The average values for frond weight and frond area, under the several experimental conditions, are set out in Tables II and III. Since the various experiments were begun with fronds of different initial weights and areas, comparisons of frond weight and area between experiments at different temperatures cannot profitably be made. The values will, however, give some idea of the effect of light at any one temperature on the frond weight and frond area; in other words, comparisons may legitimately be made horizontally in these tables, but not vertically.

TABLE II.

*Average Frond Weights.*

No. of obs.	Light intensity:—				
	Temperature.	350	750	1,000	1,400
4	15°	0.1088	0.1586	0.1454	0.1644
8	22.5°	0.0993	0.1162	0.1301	0.1170
4	25°	0.0676	0.0790	0.1017	0.1101
7	30°	0.0560	0.0775	0.1046	0.1097
6	35°	0.0501	0.0525	0.0761	0.0882

TABLE III.  
Average Frond Areas.

No. of obs.	Light intensity:—				
	Temperature.	350	750	1,000	1,400
4	15°	0.0401	0.0418	0.0447	0.0433
8	22.5°	0.0453	0.0452	0.0471	0.0436
4	25°	0.0267	0.0283	0.0276	0.0279
8	30°	0.0314	0.0341	0.0330	0.0306
6	35°	0.0264	0.0260	0.0263	0.0254

The frond weight, which is largely a measure of stored starch, increases with increasing light intensity up to 1,000 foot candles. That light has a positive effect on the frond weight, may be seen from an analysis of variance of the data of Table II given below (Table IV).

TABLE IV.

	<i>n</i> - 1.	Sum of sq.	Variance.	<i>z</i> .
Light . . .	3	0.005107	0.001702	1.37362
Temperature . .	4	0.014364	0.003591	
Errors . . .	12	0.001308	0.000109	
Total . . .	19	0.020779		

A similar analysis of the data of Table III shows that at all temperatures in this Table, the frond area is *independent* of light intensity. It may be concluded, then, that within this range of light intensities, the intensity of light does not affect the *final* area of the frond, though it does of course affect the rate of increase of area.

It has been established that within the range of exponential growth the frond area is independent of the light intensity. At light intensities between 100 and 250 foot candles, however, the frond area increases with increasing light intensity. An analysis of the areas in the experiment (Expt. 7) gives the following table:

TABLE V.

	<i>n</i> - 1.	Sum of sq.	Variance.	<i>z</i> .
Light . . .	3	0.00001604	0.000005347	1.982
Time . . .	2	0.00000215	0.000001107	
Error . . .	6	0.00000607	0.000000101	
Total . . .	11	0.00002426		

The frond weight at low light intensities is independent of the light; the probable explanation of this being that at light intensities from 100 to 250 foot candles no starch is stored in the fronds.

3. *Chlorophyll content.* In Table VI are assembled the average values

for the chlorophyll content of 100 fronds. It is clear that at every temperature the chlorophyll content is highest at 350 foot candles, and decreases progressively at higher light intensities. Table VI is bound by the same limitations as Tables II and III, in that comparisons may only be made horizontally, and not vertically.

TABLE VI.  
*Average Chlorophyll Content.*

Light intensity:—	350	750	1,000	1,400
Temperature.				
22.5°	0.033	0.030	0.028	0.026
25°	0.024	0.020	0.017	0.016
30°	0.043	0.039	0.035	0.030
35°	0.014	0.013	0.013	0.012

From an inspection of Table VI it would be expected that the rate of carbon assimilation of *Lemna* was low at 35° C., and lower at 1,400 foot candles than at 1,000 foot candles. A rough estimate of the assimilation rate may be made by dividing the increase in carbon content for the period of an experiment, by the mean area and the time. This has been done, and the results are set out in Table VII, as the apparent assimilation in mg. CO<sub>2</sub> per hour, per 100 cm<sup>2</sup> frond area.

TABLE VII.

Light intensity:—	350	750	1,000	1,400
Temperature.				
15°	1.68	4.36	4.28	3.70
22.5°	3.06	3.05	5.00	2.92
25°	2.97	4.48	8.65	8.70
30°	3.04	2.34	2.93	4.62
35°	1.35	1.54	1.53	2.45

Although the standard error of these figures is of the order of 25 per cent., there is clear indication of a lower assimilation rate at 35°, but no clear indication of a lower assimilation rate at 1,400 foot candles.

At 15°, 22.5°, and 25° fronds were removed from colonies at the different light intensities, and their respiration measured at 25° C. by a method to be described elsewhere. The respiration rates, at 25° C. of fronds grown at 15°, 22.5°, and 25° did not differ significantly. The average value was 1.063 mg. per hour per 100 cm.<sup>2</sup> frond area. The average real assimilation rate at 25°, therefore, is of the order of six times the respiration rate at that temperature.

The work was carried out at the Imperial College of Science and Technology, under the direction of Professor V. H. Blackman, to whom

I wish to record my thanks for unfailing interest and helpful criticism. I desire also to thank Dr. F. G. Gregory for helpful suggestions and criticism, and Mr. Unwin for preparing the graphs.

#### SUMMARY.

1. Colonies of *Lemna minor* were grown in a nutrient solution of constant composition, and under the combinations of light and temperature detailed on page 516.

2. The growth under these various conditions was analysed into increase of frond number, area, and dry weight. In addition, measurements were made of the chlorophyll content and respiration rate in certain of the experiments.

3. The frond numbers in the colonies at 15°–30° C., and 350 to 1,400 foot candles increased exponentially for the periods of the experiments. The relative growth rates of these colonies can therefore be expressed by the slopes of the regression lines of the logarithms of frond number on time, i.e. by the regression co-efficients. These co-efficients can be combined to give a 'light-temperature surface' for the relative growth rate of the clone of *Lemna* under observation.

4. Under the conditions of the experiments, the optimum relative growth rate of *Lemna* occurs in the region of 30° C. and 1,000 foot candles. The value of the temperature co-efficient for growth is  $Q_{10} = 2.854$ . Between 15° and 22.5° the rate of increase of relative growth rate with temperature is independent of the light intensity.

5. At 35° C., and at light intensities below 350 foot candles, the relative growth rate was no longer constant in time, but fell off more or less rapidly. Nevertheless, at supra-optimal temperatures light still has a significant effect on growth, and at sub-optimal light intensities temperature still has a significant effect on growth.

6. Within the range of exponential growth the frond area is independent of light intensity, and the frond weight increases with light intensity.

7. At low light intensities, below the range of exponential growth, the frond area increases with increase of light intensity, and the frond weight is independent of light intensity.

8. The chlorophyll content is at its maximum at 350 foot candles and at all temperatures decreases with increase of light intensity.

9. The real rate of carbon assimilation in *Lemna* is of the order of six times the respiration rate.

## APPENDIX

BY

ERIC ASHBY.

In 1928 the writer carried out experiments on the interaction of light and temperature on the growth of *Lemna* between 300 and 1600 foot candles, and at temperatures ranging from 17° to 35° C. The technique employed was the same as that used by Dr. Hicks in 1930, and the data are therefore subject to the same reservations.

Most of the phenomena described in the foregoing paper were encountered in these earlier experiments, although the strain of *Lemna* used was different from that used by Dr. Hicks. Between 17° and 30° C. and at all light intensities from 300 to 1600 foot candles the growth rate of colonies of *Lemna* was exponential. The frond area was independent of both light intensity and temperature, and the frond weight increased with light intensity.

The 1928 strain differed, however, from that used by Dr. Hicks in the position of the optimum light intensity. In this earlier strain at all temperatures the relative growth rate was still rising at a light intensity of 1600 foot candles. The behaviour of this strain in respect of frond number is summarized in the accompanying table, which may be compared with Table I in Dr. Hicks' paper.

*Regression Co-efficients of Lines of Closest Fit when Logarithms of Frond Number are Plotted against Time. The Values are given over the Range within which Growth remains Exponential.*

Light intensity :—		300	550	750	1,600
Temperature.					
17.5° C.		—	0.0508	0.0578	0.0636
	S.E.		0.00032	0.00048	0.00063
28.8° C.		0.0535	0.0646	0.0746	0.0803
	S.E.	0.00055	0.00052	0.00088	0.00072
25.7° C.		0.0706	0.0963	0.1004	0.1070
	S.E.	0.00056	0.00072	0.00092	0.00121
30.0° C.		0.0927	0.1226	0.1304	0.1362
	S.E.	0.00086	0.00135	0.00154	0.00172

Subsequent work has verified the fact that different strains of *Lemna* have different light optima when grown under the same conditions of light and temperature. The light-temperature surface outlined by Dr. Hicks is now being examined in greater detail for different strains of *Lemna*.



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# Studies in *Coprinus sphaerosporus*.

## I. The Pairing Behaviour and the Characteristics of Various Haploid and Diploid Strains.

BY

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With Plate XI and four Figures in the Text.

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### I. INTRODUCTION.

WHILE recent researches have thrown considerable light on the inheritance of a number of morphological characters in certain of the Ascomycetes—especially the work of Dodge (2), and of Lindegren (3, 4, and 5), on species of *Neurospora*—very little is so far known of the inheritance of such characters in the Agaricaceae, the large amount of work

which has been done on this group having been for the most part confined to a study of sex and incompatibility factors. Zattler (6), however, working with *Schizophyllum commune* and *Collybia velutipes* has investigated the inheritance of 'Knäuel-Fruchtkörper' in the former fungus, and that of mycelial colour in the latter. He has shown that the two types of fruit-body of *Schizophyllum* depend on a single factor difference, the 'Normal' being dominant to the 'Knäuel-Fruchtkörper', and that in *Collybia* the colour of the haploid strains is conditioned by the presence of two pairs of factors. A preliminary examination of *Coprinus sphaerosporus* revealed the presence of numerous morphological, as well as physiological differences between the various haploids, and it was with a view to investigating the inheritance of the factors influencing these that the work was begun. The present paper is concerned chiefly with the interaction of the various haploid and diploid strains on one another, as well as with the effect of X-rays in inducing saltation. It is hoped to deal with the question of the inheritance of the various characters in a later paper.

## 2. SOURCE OF MATERIAL.

At Professor F. W. Oliver's suggestion, two sun-dried mud bricks which he had brought from the Lahun Pyramid, Egypt (a pyramid composed almost entirely of such material, and situated on the confines of the Fayoum) some three months previously, were broken up, and the material of which each was composed was placed in a box and watered, with the result that in a few days' time the soil from one of the bricks was covered with mycelium which, in some two weeks from the time of its first appearance, gave rise to fruit-bodies of the *Coprinus* type. Spores from one of these fructifications were taken and sown on malt-agar in a Petri dish where they germinated and gave rise to the diploid colony known as Strain A.

The brick from which the fungus arose was an exposed one situated near the base of the Pyramid, and at a corner. A number of other bricks have since been taken from the same position, and also from a wall near the base of the Pyramid, but this fungus has not been again obtained. The fungus is not known locally either in the soil or as a contaminant in laboratory work. The bricks measured some  $42 \times 22 \times 13$  cm. and were composed entirely of soil and chopped straw, the latter being in a good state of preservation, despite the fact that the bricks are about 4,000 years old. An inch or so of soil was removed from each face of the bricks before they were broken up. Several weeks after the second lot of bricks had been collected their organic and water contents were estimated, with the result that they were found to contain on an average 4 per cent. of water, the extreme values being 3.5 and 4.2 per cent., and 5.7 per cent. organic matter, with extremes of 5.6 per cent. and 5.9 per cent. It is

probable that the amount of water in the bricks, when estimated, did not differ greatly from the content before removal from the Pyramid.

It is worthy of note that the fungus, on its first appearance, became apparent over the whole surface of the soil at the same time, and that this has also been the case with certain fungi which have appeared on the other bricks. As it would have been impossible for the fungus to have spread from one point in the soil over the whole of the exposed area (some 50 × 60 cm.) in the time available, the soil must have been inoculated in the first instance in a number of different places.

The fungus is believed to be a new species of *Coprinus*, and a taxonomic description will be given elsewhere under the name *Coprinus sphaerosporus*.

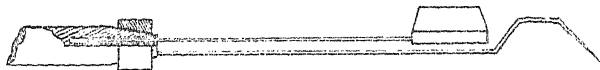
### 3. EXPERIMENTAL PROCEDURE.

Culture work was carried out in all cases on malt-extract agar (2 per cent. malt, 2 per cent. agar), unless otherwise stated. The temperature was maintained at 25° C.

Single-spore colonies were obtained in two ways. In the first of these, spores were smeared on a glass slide and then lifted one at a time on the point of a fine needle and placed on malt-agar to germinate. The alternative method was to prepare a suspension of spores in sterilized water, which was then poured into a Petri dish containing solidified malt-agar, the surplus being instantly drained off. The spores were found to germinate very satisfactorily under these conditions. The resulting colonies were examined under the microscope before being removed to a test-tube in order to be certain that each had originated from a single spore. After a little practice it was found possible to adjust the strength of the suspension, so that on pouring the plates a small number of colonies was produced on each, with the result that each colony was well separated from its neighbour. The first colonies to arise were visible to the naked eye within twenty-four hours of the spores being placed on the medium, and practically all the spores which did germinate had done so within forty-eight hours. The colonies were transferred as they became visible, so that a later-germinating colony was not overgrown by one which had germinated earlier. By this means a representative sample of the different types of haploid was obtained.

Growth-rates were determined by measuring the increase in diameter of a colony growing on malt-agar in a Petri dish. A cutter (Text-fig. 1) was used to ensure that all inocula were of equal size. It consisted of a cylinder of hard steel with an internal diameter of 8 mm. turned to give a cutting edge at one end. This was attached to the handle of an inoculating needle by means of nickel chrome wire. The needle itself was also of nickel chrome wire, and was mounted in the holder beside the cutter.

Two holes were drilled in the holder to take the wires, and the end of the holder was sawn in two in the plane of the holes. In the figure part of the holder is shown sectioned in the plane of the saw cut. The wires were held in place by a brass ring which was forced over the tapered end of the



TEXT-FIG. I.

holder. This arrangement was found to be very satisfactory in use, as well as being more robust and easier to make than the more usual method of screwing the ring on to the holder. In use the cutting cylinder was pressed down into the medium as far as the top of the tapered portion and then withdrawn, leaving the disc of agar still in place. The needle was then used to cut the disc free and remove it. The needle was bent as shown to facilitate the extraction of inocula from narrow test-tubes. Mounting the cutter and needle on the one holder enabled both to be sterilized at the same time, and to be inserted into the test-tube or Petri dish together, thereby considerably reducing the chance of contamination, and appreciably curtailing the time required in effecting a transfer of medium.

Petri-dish cultures were always employed for X-ray work. The colony to be irradiated was allowed to grow till it had reached the required size—in most cases till it had completely filled the dish—when the glass lid of the dish was replaced by one of celluloid and the culture exposed to the rays. Following irradiation a number of inocula were taken from the culture and placed each in a separate dish. Where a large number of cultures had to be X-rayed at the same time (such, for example, as the irradiation of 150 strains described in Section 8), a set of cells was prepared from celluloid cemented to a glass base, and the whole covered with a celluloid lid. Each culture to be X-rayed was placed in a different cell. Only haploid cultures were irradiated, and as these produce no spores the chances of one colony contaminating adjacent ones were reduced to a minimum. No evidence of any such contamination was obtained. The X-ray tube used was of the Coolidge type, and was run at 90–100 K.V. with a tube current of 4 milliamps. The cultures were exposed at a distance of 30 cm. from the target, and were irradiated for three hours. Apart from the celluloid screen no other filter was employed.

Saltants arising from X-rayed material were purified by the hyphal-tip method.

The following scheme of reference to successive generations and to different strains has been adopted. The symbols  $F_1$ ,  $F_2$ , &c., have been used in the ordinary sense, except that they apply to succeeding haploid generations in place of the usual diploid. The first diploid colony obtained

in pure culture is referred to as strain A, as already stated. The  $F_1$  generation of haploid colonies derived from basidiospores produced by strain A were numbered consecutively from 1 to 30 on being isolated, and are referred to by these numbers. Saltants derived from any of these haploids were given a second number, thus 5,7 refers to saltant number 7 derived from strain 5. When two haploids A and B are paired two diploids may arise, one consisting of the cytoplasm and nuclei of A diploidized by the nuclei of B, the other consisting of the cytoplasm and nuclei of B diploidized by the nuclei of A. To distinguish these two in, for example, a cross between strain 5 and strain 12, the symbol  $5 \times 12$  will be used to represent the mycelium of 5 diploidized by 12, and  $12 \times 5$  to represent the mycelium of 12 diploidized by 5. When no note has been made as to which diploid strain is in use, the symbols  $5 \times 12$  or  $12 \times 5$  are used indiscriminately. In the same way strain 5, diploidized by the diploid  $5 \times 12$ , is represented as  $5/5 \times 12$ .

#### 4. GENERAL CULTURAL OBSERVATIONS.

*Coprinus sphaerosporus* has been found to be very convenient for work on account of the readiness with which it fruits in pure culture. Strain A has been grown on dung, and on a mixture of dung and soil, and while it fruits readily on both of these media, it has been found more convenient during the course of these experiments to grow it on malt-agar. On the latter medium fruit-bodies are freely produced, whether the fungus is grown as a Petri-dish culture, or on a test-tube slope, the only difference being that in the former case the fruit bodies are on the whole larger than in the latter. Owing, however, to the greater freedom from contamination of the test-tube method of culture, this has been adopted throughout, except on rare occasions. Light was not found to be necessary for the production of mature fruit-bodies as is the case with *C. lagopus*. The above remarks apply to strain A. In the case of the other diploid cultures only the malt-agar slope method has been used, and as will be shown in Section 5, definite differences have been established for this medium in the degree of maturity reached by the fruit-bodies of the various strains.

The frequency with which clamp-connexions are produced has been found to vary considerably from one diploid to another. In some strains they occur at practically every transverse wall, while in others they are rare, and are only to be found with difficulty.

While the haploid mycelia differ markedly from one another in their general appearance in culture, the diploids from whatever source they may be derived are very similar, in that the aerial mycelium is relatively thin, and although the individual hyphae are grouped into thin rhizomorph-like strands (which is very rarely the case with the haploids), the surface in general has a very even density of mycelium. In some strains circles of

mycelium, denser than that of the rest of the colony, are found, e.g. in strain 12 × 5 (Pl. XI, Fig. 10), and in most cases fruit-bodies arise from the neighbourhood of these rings. The number of rings is generally five (Pl. XI, Fig. 13), in some cases, however, no rings are produced (Pl. XI, Fig. 11). Fruit-bodies, as well as arising from the rings of denser mycelium, also occur at the edge of the colony, and in not a few cases actually on the glass beyond the medium. Strain A has now been in culture on malt-agar for upwards of a year, and still fruits as freely as on its first appearance. No other diploid produces fruit-bodies as plentifully, and in most cases the spores liberated by each fruit-body are much less numerous than with this strain.

Thirty haploid strains derived from a fruit-body of strain A were selected at random and grown on horse-dung extract agar. On this medium the aerial mycelium was in all cases very thin. It was found possible, however, to separate the thirty strains into two groups in which the members of one group develop a more plentiful aerial mycelium than those of the second, the difference in the amount of aerial mycelium being greater in young cultures than in old. The former group contained thirteen and the latter seventeen strains. On malt-agar it was not found possible to effect a definite segregation of all the strains into two groups, as on this medium other differences between the individual strains became apparent, and tended to mask that of the amount of the mycelium. After the thirty strains had been paired (*vide* Section 5), it was found that the thick and thin types of mycelial growth were related to the pairing properties of the different strains, the aerial mycelia of the strains of one pairing group being more plentiful than those of the members of the other group. Actually of the thirty strains fifteen were found to belong to each pairing group, so that had the amount of mycelium been used as a criterion of pairing, only two of the thirty strains would have been placed in the wrong group. These were strains 1 and 7 which were placed in the 'thin-mycelial' group, whereas according to their pairing reactions they belonged to the group having a more plentiful aerial mycelium.

## 5. THE RESULTS OF PAIRING THIRTY HAPLOID STRAINS.

Cultures each arising from a single spore of a fruit-body of strain A were segregated, and thirty selected at random. These were numbered 1 to 30 consecutively, and were divided arbitrarily into four groups, two of seven and two of eight cultures each. Each strain in any one group was then paired with all the other strains in the same group, and also one (or two, if necessary) strain from each group taken in turn was paired with all the strains of the following group. Pairing was effected by placing two inocula, one from each member of the pair, together in the same test-tube



and incubating at 25° C. for three weeks. In this way the pairing properties of each strain were determined, and it was found possible to arrange the strains into two groups, each containing fifteen strains, on a basis of their positive reactions, i.e. the production of perfect fructifications. Strain 1 differed from the others, in that it did not give perfect fruit-bodies with any other strain, it did, however, produce fructifications when paired with strains 4 and 30, which were perfect except for the absence of spores, and has therefore been placed in the opposite group to that to which these strains belong.

An examination of the cultures containing diploid mycelia revealed well-defined differences, in the readiness with which fruit-bodies were produced, and in the degree of perfection attained by the various fructifications when formed. In the case of strain A the average test-tube culture produced some six or seven fruit-bodies, nearly all of which matured and liberated a large number of spores. In the case of the above pairings, however, while some of the cultures contained numerous mature fruit-bodies, others produced none at all on mycelia which otherwise appeared to be typically diploid. Intermediate stages were also of frequent occurrence, in that some strains gave rise to small sessile fructifications which never elongated their stipes, and the pilei of which never opened, while others produced fructifications with long stipes but small undeveloped pilei. A third type which occurred more rarely than any of the others consisted of fruit-bodies which, while otherwise perfect, failed to give rise to spores, or only produced them very sparingly.

TABLE I.

Strain No.	16	13	12	29	24	19	10	22	30	3	4	25	28	23	20
14	P	P	P	P		B	P								
17	P	P	P	B	P	B	P		C			C			
26	C	P	A	P	B	B	B		A			C			
5	P	B	P	P	B	B	B	P	B	P	B	P	C	C	
9	P	P	P	C		A	P	C	P	P	P		P	P	P
11	P	P	P	P	C	P	P		A	C			A	B	
8	C	B	B		C	C		C	C	B	P		B	P	P
15			C			P	A			C	P		P	P	A
2		C		C			P		A	P	P				
7	C			C			P		B	P	P		P	C	C
21					B	P	P				A			B	C
27	C			C		B	P		A				B	A	A
18	C					B	B	B	P	P	A		B		
6										P			B		
1			B	C					A	B	A				

It was thought worth while to see if there was any regularity in the occurrence of these types between pairings of the various strains, and whether any correlation could be established to explain their appearance. To do this each of the fifteen strains of one group was paired with every

member of the other group, and the type of fructification noted in each case. The results are set out in Table I, in which the significance of the various letters which refer to the type of fruit-body is as follows:

*P* represents a perfect fructification with long stipe, expanded pileus, and plentiful black spores.

*A* represents a fructification with long stipe and expanded pileus, but with very few, or more commonly, no spores.

*B* represents a fruit-body with long stipe and unopened pileus (generally very small).

*C* represents a sessile fructification with small unopened pileus.

Where no letter occurs in the table no fruit-body of any kind was produced. In the latter case the mycelium in nearly all cases showed strands of grouped hyphae, typical of the diploid, and on examination proved to have clamp-connexions, though in some cases these were very rare. Occasionally, however, an entirely negative result was obtained, the two haploid mycelia being present, together with no sign of diploidization having occurred.

Where the fruit-body produced was not perfect, and also when no fructifications occurred the pairing was repeated, and on a similar result being obtained, repeated a second time. In this way all the pairings represented by the table, with the exception of the *P*'s, were carried out in triplicate. In most cases the results were not affected by repetition, but where a different result was obtained, the most perfect fruit-body produced has been quoted as representative of the pairing.

The numbers of pairs found in each of the five groups were as follows: 55 in group *P*, 15 in group *A*, 29 in group *B*, 28 in group *C*, and the remainder, namely 98, with no fructifications.

It appeared possible that the failure to mature perfect fruit-bodies in some pairings might have been due to some change in the haploids during the time they had been in culture before the tests were completed, a period of some four months. In order to see if this was the case a number of pairings which had originally given perfect fructifications were repeated about eight months after the haploid strains had been segregated or four months from the time the last pairings had been made. Altogether eight repeat pairings were made and in each case mature fruit-bodies were produced, so that there is no reason to believe that failure to pair in the first place was due to deterioration of the fungus in pure culture.

## 6. DESCRIPTION OF VARIOUS HAPLOID STRAINS.

The thirty haploid strains differed from one another both in appearance and growth-rate. While some formed a loose textured aerial mycelium others produced a closely woven web of hyphae. In some cases the aerial

mycelium was very freely formed, while in others it was scanty. These four characters, combined with the evenness or otherwise with which the mycelium was produced on the surface of a colony, gave rise to many different morphological types. The following descriptions are of typical examples of the various types of colony obtained.

Strain 4 (Pl. XI, Fig. 1) was characterized by a thin aerial mycelium of a fairly uniform distribution. Strain 12 (Pl. XI, Fig. 3) produced a plentiful aerial mycelium arranged in very regular concentric zones. Strain 21 (Pl. XI, fig. 5) had a thin and very evenly disposed young mycelium over which in the older parts of the colony a thick mycelium spread itself irregularly. Strain 13 (Pl. XI, Fig. 2) produced an evenly dense and closely compacted mycelium with a narrow band of thin mycelium at the edge of the colony. In strain 18 (Pl. XI, Fig. 6) the aerial mycelium was freely produced and was characterized by its occurrence in broken concentric zones of denser mycelium, giving a very characteristic 'dappled' appearance to the colony. Strains 5, 8, and 10 (Pl. XI, Figs. 9, 8, and 4 respectively), were all rapid growers and in each case produced an even aerial mycelium. In strain 8 the mycelium was relatively thin, in 10 somewhat more plentiful, and in 5 very freely produced. A characteristic of strain 5 is the production of a peripheral band of very thin mycelium, generally about 0.5 cm. wide.

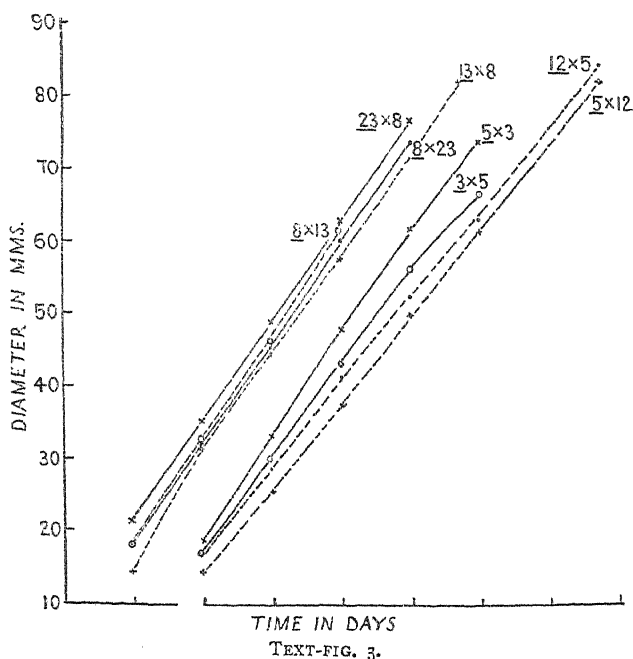
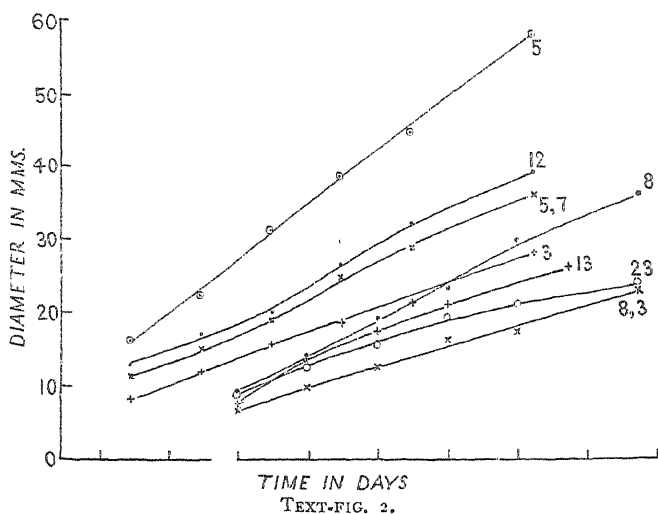
Of those strains in which the aerial mycelium is not evenly disposed over the surface of the colony two types can be distinguished. The first of these consists of those in which broken or entire concentric zones of denser mycelium occur, as in strains 18 and 23. Various degrees of brokenness of the zones were found, some strains having almost entire zones, e.g. strain 12, while in others they were broken up very completely. Seven strains of the thirty examined showed this zoning effect, three belonging to one pairing group and four to the other. In the second type of mycelium with irregular distribution no zoning of any form was found, strain 21 is typical of this group.

Some strains excreted drops of a yellowish-brown liquid in the older parts of the colony, two such drops are to be seen in strain 21 (Pl. XI, Fig. 5). In a few strains chlamydospore-like bodies were found, these arise at the margins of old colonies, but are not of frequent occurrence.

#### 7. GROWTH RATES OF VARIOUS STRAINS.

The growth rates of the various strains are represented in Text-figs. 2, 3, and 4, in each of which the ordinate represents the diameters of the colonies while the abscissa is divided into units each representing one day. Measurements of each strain were begun on the second day from the time of planting and were carried out on each succeeding day (or part of a day

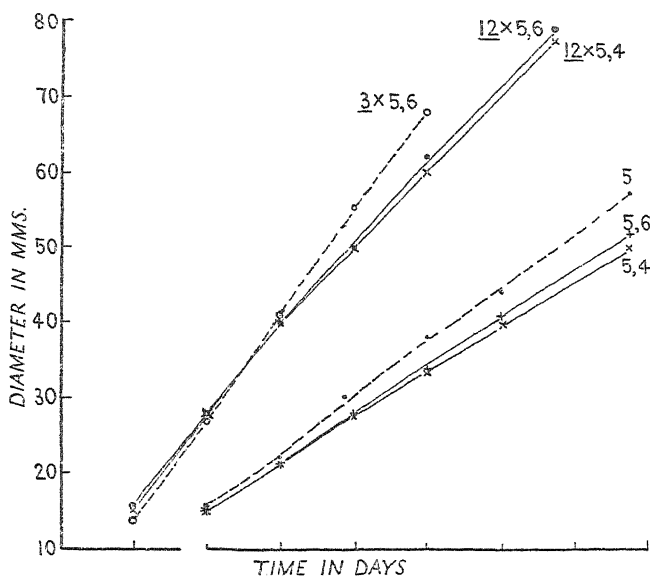
in some cases). In order to avoid confusion of the different lines in each figure the first measurements of about half the strains have been placed



one time division to the right of the remaining strains, and similarly with succeeding measurements. Thus, in Text-fig. 2, for example, measure-

ments of strains 8, 13, 23, and 8,3 are situated one unit to the right of those of strains 5, 12, 5,7, and 3 of the same age.

The experiments were carried out in quintuplicate, each point on the graphs representing the mean of the diameters of five colonies.



TEXT-FIG. 4.

(a) *Haploid*.

The growth rates of strains 5, 12, 8, 3, 13, and 23 are shown in Text-fig. 2, and it will be seen that they exhibit a wide range, strain 5, for example, growing nearly 2.5 times as fast as strain 23. Some of the growth rates are relatively constant from day to day as, for example, that of strain 5, while others show an initial increase in the rate followed later by a decrease. None of the strains have shown strong staling tendencies on malt-agar, though it is not unlikely that the dappled appearance of certain strains is a result of mild staling in a special form.

Cultures represented in Figs. 1 to 6 of Plate XI are approximately of equal age and show some variation in growth rate. The culture in Fig. 8 is somewhat older than these and that of Fig. 9 younger.

(b) *Diploid*.

An examination of Text-fig. 3 reveals a striking similarity between the growth rates of the various diploid strains despite the range in the growth rates of the haploids from which they have arisen, and a comparison of Text-figs. 2 and 3 shows that the diploids have a much greater growth rate than any of the haploids.

A comparison of the growth rates of the two diploids arising from each pair of haploids reveals very little difference between them. Strain  $23 \times 8$  has a growth rate almost identical with that of  $8 \times 23$ , and similarly with the diploids  $5 \times 12$  and  $12 \times 5$ .  $8 \times 13$  has a growth rate slightly greater from the third day onwards than  $13 \times 8$  and  $5 \times 3$  is a little faster than  $3 \times 5$ . In each case the faster strain arises from the diploidization of the faster by the slower of the two haploids. In view, however, of the smallness of the difference, and taking into consideration the identity of the growth rates of the two diploids arising from each of the other two pairings, there is no reason to believe that the difference in the growth rates of the two diploids arising from the union of two haploids is significant.

The average of the growth rates of strains  $8 \times 13$  and  $13 \times 8$  is almost identical with the mean of the growth-rates of  $8 \times 23$  and  $23 \times 8$  and also of  $3 \times 5$  and  $5 \times 3$ . These average growth-rates are slightly greater than the growth-rates of the diploids of the  $5 \times 12$  pairing, though the growth-rates of both 5 and 12 are greater than are those of any of the other haploids. In view of this there does not appear to be any positive correlation between the differences in the growth-rates of the various haploids and the growth-rates of the diploids to which they give rise.

### 8. THE EFFECT OF TREATMENT WITH X-RAYS.

In general the different strains of this fungus have exhibited little or no variation, saltation having been observed in only a few of the haploids and in none of the diploids. On subculturing the stock haploid strains after they had been in culture some six months it was observed that two strains had altered in character. Each had given rise to a dense hyphal mat lying close to the surface of the medium, while originally both had produced a relatively loose and plentiful aerial mycelium. Three months after the change had been first observed each strain was found to have continued true to its new form.

The stability of the fungus under the influence of X-rays was tested, fifteen strains being irradiated, seven of which belonged to one pairing group and eight to the other. Wide differences were found to exist in the relative frequency of saltation of the different strains. The saltants arose in all cases as sectors of varying shapes, arising in some cases at the centre of the colony, while in others they only appeared when the culture had reached a diameter of several centimetres. In some strains, such as 5 and 10, saltants were found in every plate, while in others, e.g. 12, 15, and 21, none were produced. The majority of strains produced various types of saltant. Some saltants had a denser and some a thinner aerial mycelium than the parental type. In a number of cases the growth-rate was greater than that of the parent, with the result that the arms of the sector curved away from

one another; in others the growth-rate was smaller, so that the parental mycelium gradually surrounded the saltant (in the latter case the slow growth-rate of the saltant must have been a secondary effect, probably due to staling in some form, as otherwise it would not have been able to establish itself in the first instance).

Strain 5 was remarkable in two ways. In the first place, it gave rise to diploid saltants in about half the plates, as well as to the usual haploid ones (this was verified on three separate occasions), and, secondly, the general appearance of the non-saltated part of the colony was altered. The diploid saltants arose as rapidly growing sectors, with the result that the lines of junction of the saltant with the parental mycelium curved away from one another on each side of the saltant (Pl. XI, Figs. 12 and 14). The mycelium of the diploid saltants was of the typical diploid type covering the surface of the colony evenly and with hyphae arranged in radial rhizomorphlike strands (Pl. XI, Fig. 15). The fruit-bodies were very freely produced, arising in well-defined rings, but they never developed to maturity, a stout stipe being generally produced surmounted by a small undeveloped pileus. The single ring of fruit-bodies shown in Fig. 15 is very typical of this strain and generally arose at about the same position in every culture. Clamp connexions were produced in the diploid saltants. Following X-ray irradiation the unsalted mycelium was much less plentiful than that of an untreated culture (compare Pl. XI, Fig. 9, with Figs. 12 and 14). Subcultures of this mycelium were made; and during three transfers extending over a period of a month it was found gradually to recover until it had completely regained its former appearance. Such an alteration in the appearance of unsalted mycelium was not observed in any of the remaining fourteen strains which were irradiated.

In Text-fig. 2 the growth-rate of the haploid saltant 5.7 is given, which shows it to be considerably less than that of 5. In the same figure the saltant 8.3 shows a similar relationship to its parent, strain 8. In Text-fig. 4 the growth-rates of strain 5 and those of its two diploid saltants 5.4 and 5.6 are given, and it will be seen that while both saltants have a similar growth-rate they grow more slowly than their haploid parent. This is in marked contrast to the behaviour of normally produced diploids in which the growth-rate is very much greater than that of the haploids. As already stated, the diploid saltants must grow more rapidly than their parent on their first appearance as otherwise the arms of the saltant sectors would not have diverged from one another at an increasingly larger angle. It must therefore be concluded that not only was an alteration in the appearance of the non-saltating portions of 5 produced by X-rays, but that the growth-rate was slowed down as well.

An attempt was made to discover whether the capacity of producing diploid saltants was inherited. Strain 5 was paired with 12 and 150

single-spore colonies obtained from the resulting fruit-bodies. The 150 strains were irradiated and each was subcultured on to 5 Petri dishes or large test-tube slopes. None of the resulting 750 colonies had diploid sectors, though haploid ones were freely produced. It must therefore be concluded that the capacity to produce diploids is either not inherited, or else a number of factors are concerned in its inheritance.

## 9. THE RESULTS OF VARIOUS PAIRINGS.

### (a) *Haploid with Haploid.*

In order to see whether the two diploids produced on the union of two haploids gave rise to fruit-bodies reaching a similar degree of maturity, six pairings were made as follows:  $3 \times 5$ ,  $5 \times 12$ ,  $4 \times 8$ ,  $8 \times 13$ ,  $8 \times 23$ , and  $4 \times 21$ . The first two of these produced perfect fruit-bodies on each of the four diploid mycelia, thus in the case of the cross  $5 \times 3$ , strains  $5 \times 3$  and  $3 \times 5$  gave rise to perfect fruit-bodies and similarly with the other pairing. The crosses  $4 \times 8$  and  $8 \times 23$  each produced one diploid which gave perfect fructifications and one with imperfect ones.  $8 \times 4$  and  $23 \times 8$  gave perfect fructifications while  $4 \times 8$  and  $8 \times 23$  were imperfect in that in both cases the most mature fruit-bodies consisted of a short stalk and small unopened pileus. None of the four diploids from the pairings  $8 \times 13$  and  $4 \times 21$  yielded perfect fructifications. In the case of each pairing the two diploids produced fruit-bodies reaching a similar degree of perfection, the  $8 \times 13$  fructifications consisting of a stipe and a small unopened pileus, and those of the  $4 \times 21$  cross of a stipe and opened pileus which, however, produced no spores.

In order to ascertain the pairing properties of the various saltants obtained by treatment with X-rays three saltants were selected on the grounds that each should show the greatest possible divergence from its parent. The three saltants selected were 3,2, 5,7, and 8,3, each of which has a much less dense aerial mycelium and a slower growth-rate than its parent. Pl. XI, Figs. 7 and 9 show the types of growth of the saltant 5,7 and of its parent strain 5 respectively. Each saltant was paired with two strains with both of which its parent produced perfect fructifications. Thus 3,2 was paired with 6 and 7, 5,7 with 3 and 12, and 8,3 with 4 and 23. With the exception of  $5,7 \times 3$ , both strains of each pair produced diploid mycelia. In the case of  $5,7 \times 3$ , strain 3 became diploidized while 5,7 remained haploid. The types of fruit-bodies produced by the different strains are tabulated in Table II, from which it will be seen that fewer of the diploidized saltants produce perfect fructifications than is the case of the diploidized non-saltated strains.

When two haploids are paired together it was found that there is a considerable difference in the readiness with which one member is diploidized



by the other. Four pairs were examined from this point of view and it was found that in general one mycelium is diploidized shortly after the two colonies meet, whereas the second is not affected till several days later. Of the eight strains examined the member of each pair which had the thinner aerial mycelium (and in the particular cases, a slower growth-rate), was in each case the first to be diploidized. Another difference was that the first strain to be diploidized was affected at numerous points all round the periphery at about the same time whereas the other member of the pair became diploid at only a few scattered points. The variation in the period from the planting of the two strains till the diploidization of the strain, which was the slower of the two in becoming diploid, was considerable. For example, in the cross  $8 \times 23$ , 8 was always the last to be diploidized. In four out of five plates of this cross, all of which were the same age, strain 8 was still haploid and had an average diameter of 6.1 cm., whereas in the fifth plate 8 had been diploidized when only 3 cm. in diameter.

TABLE II.

Pairing.	Strain.	Fruit-bodies.	Strain.	Fruit-bodies.
$8,3 \times 4$	8,3	imperfect	4	imperfect
$8,3 \times 23$	8,3	imperfect	23	perfect
$3,2 \times 6$	3,2	perfect	6	perfect
$3,2 \times 7$	3,2	perfect	7	perfect
$5,7 \times 12$	5,7	imperfect	12	imperfect
$5,7 \times 3$	5,7	not diploidized	3	perfect

A comparison of the mycelial characters of the pairs of diploids produced by pairing various haploid strains has shown that it is impossible to separate one member of each pair from the other, thus, for example,  $8 \times 23$  is indistinguishable morphologically from  $23 \times 8$ . It was found, however, that there are well-defined and constant differences between the diploids resulting from one pair of haploids and those derived from another. Thus it was found that in those strains in which rings were produced, namely,  $8 \times 23$ ,  $5 \times 12$  (Pl. XI, Fig. 10), and  $3 \times 5$ , although the number of rings was the same in each case, there was a difference in the definition of the rings between the several pairs, some having more clearly marked rings than others. The amount of colour produced in the older parts also showed a small but definite difference, in this respect  $8 \times 23$  was the most coloured and  $5 \times 12$  the least. The four diploids of  $8 \times 13$  (Pl. XI, Fig. 11), and  $4 \times 21$  were different from the remainder in that no rings were produced in any case and there was no colouring of the mycelium. As these two crosses do not produce perfect fruit-bodies, whereas the remainder do so, and since fruit-bodies are in general associated with the rings, it is possible that there is some connexion between these two facts.

(b) *Haploid with Diploid.*

In order to test the reaction of a diploid strain on a haploid one, eight haploid strains were taken and combined to give four pairs, each of which in turn gave rise to two diploid strains. Every diploid was then paired in turn with the two haploids from which it had been derived in the first place, and the latter were examined to see whether they in turn were diploidized. For example, strains 3 and 5 were paired and gave rise to the diploids  $3 \times 5$  and  $5 \times 3$ , each of which was then paired with 3 and 5 in turn. It was found necessary, owing to the slower growth-rate of the haploids, to plant them some time before the diploids with which they were to be paired. It was found in all sixteen cases that the haploid strain was diploidized by the diploid one, so that in the above example, for instance, the four diploids  $5/5 \times 3$ ,  $5/3 \times 5$ ,  $3/3 \times 5$ , and  $3/5 \times 3$  were produced.

Differences were observed in the reactions of the various strains with one another. In all cases the diploid owing to its greater growth rate spread itself round part of the circumference of the haploid. In the majority of cases the complete surrounding of the haploid was prevented by its becoming diploid at numerous points along the free circumference, the points growing out to form small fan-shaped sectors before their different mycelia fuse to form a more even edge to the colony. In a few cases, e.g.  $23/8 \times 23$ , however, the diploidization of 23 started at its point of contact with  $8 \times 23$  and proceeded regularly from this point round its periphery. The two diploid mycelia  $8 \times 23$  and  $23/8 \times 23$  were identical in appearance and had similar growth rates. It was found, however, that the distance from the point of inoculation of  $8 \times 23$  to the most advanced point in the diploidization of 23 was greater than the radius of  $8 \times 23$  where its advance was not affected by the presence of 23 or  $23/8 \times 23$ . It therefore appears that the diploidizing effect spreads more quickly than the resultant diploid grows.

The appearances of the pairings  $23/8 \times 23$  and  $23/23 \times 8$  were identical, as also were  $8/8 \times 23$  and  $8/23 \times 8$ . The results of pairing 8 with  $23 \times 8$  were, however, different from those arising from the 23 by  $23 \times 8$  cross. When 23 was the haploid it gradually became diploid round the circumference, starting from its point of contact with  $8 \times 23$  as already described, when 8 was the haploid, however, diploid mycelia first appeared at various points on its free circumference and only joined up with another and with the original diploid later. These relationships were found to hold good with other strains, so that where a haploid is paired with a diploid it is the haploid which determines the type of the reaction and not the diploid.

In the above examples it was found that in all eight cases a haploid could be diploidized by another colony the product of itself diploidized by another strain. In the case of the diploid saltants 5,4 and 5,6, however,

they were not found to be able to diploidize their parent, strain 5. Each saltant was paired with 5, 3, and 12 (five pairs with 3 and 12, producing perfect fruit-bodies in each case), and after a period of growth each culture was examined to see which of the haploids, if any, had been diploidized. In the four cases resulting from the union of 3 and 12 with the two diploid saltants taken in turn it was found that both 3 and 12 had become diploid and had produced perfect fruit-bodies. The two mycelia in each of the pairings  $5,4 \times 5$  and  $5,6 \times 5$  had no apparent effect on one another, the two haploids remaining haploid. The diploids  $12 \times 5,4$  (Pl. XI, Fig. 13) and  $12 \times 5,6$  were identical and similar to  $12 \times 5$ . In the same way  $3 \times 5,4$ ,  $3 \times 5,6$ , and  $3 \times 5$  were identical ( $3 \times 5$  differed from  $12 \times 5$  in that the rings of denser mycelium were better defined and there was a more intense coloration of the mycelium).

#### 10. DISCUSSION.

Since the brick must have been inoculated with the fungus at a number of different places, and as *Coprinus sphaerosporus* is unknown as a local contaminant, it appears certain that the brick was already infected when obtained from the pyramid. How long the fungus had been present in the brick, in what form it was present, and in what manner infection occurred in the first instance remain, however, open questions.

Strain A has never been cultivated from a single hyphal-tip, so that it is not known whether it consists of a single diploid mycelium derived from the union of two spores, or whether it is a mixture of such mycelia. Several diploids each derived from the union of two haploids have been examined, and each was found to yield spores which gave rise to colonies differing from one another in the characteristics of their aerial mycelia and in growth rate. In this respect therefore strain A is comparable with any experimentally produced diploid which has been examined. It differs, however, from any other diploid in the extreme freedom with which sporophores are produced, and should it be composed of a mixture of different diploids its high fertility might be the expression of an increased vigour resulting in some way from such a mixture. On the other hand, it may well be that the fungus is becoming weakened by cultivation on artificial media, and that this process is more rapid in the haplo- than in the diplo-phase (in support of which it may be pointed out that the haploids produce saltants occasionally, whereas none of the diploids have been seen to do so). If this were so, then all the diploids might be expected to show less vigour than strain A, as this is the only one not derived from haploids grown in pure culture.

It has been possible to arrange all the haploid mycelia examined in two groups so that any member (with the exception of strain 1) of one group produces perfect fruit-bodies with one or more members of the other

group. In addition to the production of perfect fructifications, several types of imperfect sporophores are formed on pairing certain members of the opposite groups. Altogether 60 intra-group pairings have been made, and in no case have sporophores or a diploid mycelium been produced. Pairings within the groups were carried out at random and, while far from complete, they indicate that the strains of either group are almost certainly sterile when paired together. The unpaired haploids have also during nine months' culture proved to be completely self sterile. The arrangement of the haploids into two groups on a basis of their pairing properties is to be correlated with their segregation into two (practically) identical groups on morphological grounds, the strains of one pairing group being found to possess a more plentiful aerial mycelium than those of the other. While the pairing properties combined with the mycelial characteristics of the haploids indicate a single factor difference for these characters, an attempt to explain the production of various degrees of imperfection in the fructifications on a factorial basis has been unsuccessful. While it appears reasonable to regard the single factor associated with the pairing properties and mycelial production as a sex factor, an adequate interpretation of the several degrees of fertility exhibited by the various diploids is at present impossible. If sterility (or incompatibility) factors are present in addition to the sex factor and are responsible for the variations in the degree of fertility exhibited in certain cases, they must be numerous (too numerous to be capable of analysis from the available data) and must affect not only the degree of fertility of the diploid when formed, but must also, in those cases in which the diploid was not produced, condition the fusion of the haploids.

The two diploids arising from the union of two haploids are exactly similar morphologically and have in most cases the same growth-rate, although in others the diploidized faster haploid may spread a little more rapidly than the diploidized slower one. The two haploids resulting from each of certain pairings, however, have been found to give rise to sporophores which reached different degrees of maturity. For example,  $8 \times 4$  produced perfect fruit-bodies whereas  $4 \times 8$  gave rise to imperfect ones. In cases such as these the different types of reaction must be due either to a difference in the cytoplasm or to a difference in the nuclear content of the two diploids. Since the nuclei of both diploids are derived from the same haploids and presumably retain their identity unchanged in the diplophase, the only possible difference between the nuclei of the two diploids appears to lie in the relative numbers of nuclei from each haploid. Whether such a difference exists, or whether all the nuclei of a diploid occur in pairs, one member of each pair being derived from one and the other from the second of the two haploids is not known.

Diploids arising from the union of different haploids are dissimilar to one another in appearance, they have, however, almost identical growth-

rates irrespective of the growth-rates of the haploids from which they were derived. This anomaly would be explained if the factors controlling growth-rate in the diplo-phase were not the same as those responsible for rate of spread in the haplo-phase, and if all the haploids carried the same factor or factors controlling growth-rate in the diploid. An alternative suggestion is that the stimulus following fusion and the production of the diplo-phase is such that the influence of the growth-rate factors supplied by the haploids is only of secondary importance, and may be responsible merely for such small differences as have been observed in a few cases between one diploid and another. The greatly increased growth-rate of the diplo-phase over that of the haploid lends support to the latter hypothesis.

As described in Section 9 (*a*), when two haploids meet they are not as a rule diploidized at the same time or in quite the same manner. In all the cases examined it is noteworthy that the strains which were the first to become diploidized belonged to one sex and had a thinner aerial mycelium and slower growth-rate than those which became diploid later on. In Section 9 (*b*), where diploids were paired with haploids two types of reaction are recorded. Buller (1), in his experiments on *C. lagopus*, found that he got two different results when he made 'legitimate' and 'illegitimate' pairings between haploids and diploids. In the former case (e.g. when he paired the haploid (*ab*) with the diploid (*AB*) + (*ab*)), he found that the haploid became diploidized regularly all round its circumference, whereas on making an 'illegitimate' pairing, such as (*ab*) with (*Ab*) + (*aB*) the haploid was in some cases diploidized only part of the way round its circumference, while in others, although it became diploidized all round, the diploid mycelium occurred irregularly in patches. In the present instance it is not possible to form 'illegitimate' pairings as there are only two sex groups and both types of reaction would belong to the 'legitimate' class. It is of interest, however, to note the two types of behaviour which occur in each fungus, although it is impossible to make a satisfactory comparison of one with the other.

## II. SUMMARY.

The fungus was obtained from a sun-dried mud brick taken from the Lahun Pyramid in Egypt, and is believed to be a new species.

Thirty haploid mycelia were obtained from spores derived from a fruit-body produced by strain A—the first strain to be obtained in pure culture. These differed widely from one another in both morphology and growth-rate. On pairing the haploids each was found to belong to one or other of two groups of which every member of one group produced fruit-bodies with one or more members of the other group. Half of the thirty strains belonged to one group and half to the other.

While some of the diploids produced by the union in pairs of the

different haploids gave rise to perfect fruit-bodies, the majority produced only imperfect ones, and in some cases no sporophore was formed at all. In certain instances also no diploid mycelium was produced on pairing haploids from the opposite pairing groups. It is considered that the two pairing groups are due to a single factor difference, but the evidence is not sufficient to show whether the various degrees of sterility exhibited are due to the presence of incompatibility factors and, if so, how many such factors are concerned.

Measurements are given of the growth-rates of both haploid and diploid strains, and it is shown that while the various haploids exhibit wide differences in growth-rate the diploids, irrespective of their origin, are very uniform in this respect, and have a rate of spread much greater than that of any of the haploids. A comparison has been made between the two diploids arising from the union of two haploids, but while they are found to be practically identical in both growth-rate and appearance, in some cases one gave rise to perfect fruit-bodies and the other to imperfect ones. It is concluded that the difference in the degree of perfection of the fructifications must be due either to the influence of the cytoplasm, or that the relative numbers of nuclei supplied by the two haploids must differ in each of the diploids produced by their union.

A description is given of the several types of diploid mycelia produced by the union in pairs of the different haploids.

Saltants arising as a result of X-irradiation are described and their fertility when paired with unirradiated haploids recorded. Strain 5 was found to be unique in that it gave rise to diploid saltants following irradiation; these produced imperfect fruit-bodies, and the mycelium, although morphologically similar to that of a normally produced diploid, had a considerably smaller growth-rate. To examine the inheritance of this X-ray effect strain 5 was paired with strain 12, and 150 single-spore colonies derived from spores produced by the resulting diploid were X-rayed, but in no case were diploid saltants produced.

On pairing one haploid strain with another a difference was found in the rapidity and manner with which each became diploidized, and it was established that the strains of one sex were generally diploidized earlier than those of the other.

Two types of reaction between a haploid and a diploid mycelium are described, in one case the haploid becomes diploidized regularly round its periphery, starting from the point of contact with the diploid, while in the other the haploid gives rise simultaneously to diploid mycelia at numerous points along its free circumference.

I have very great pleasure in thanking Professor F. W. Oliver not only for providing the material from which the strain of *C. sphaerosporus*

was obtained but also for his advice and unfailing help throughout the course of the experiments.

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#### EXPLANATION OF PLATE XI.

Illustrating Dr. Hugh Dickson's paper on 'Studies in *Coprinus sphaerosporus*. I. The Pairing Behaviour and the Characteristics of Various Haploid and Diploid Strains'.

The photographs were taken by reflected light only.

Fig. 1. Strain 4. This strain has a thin aerial mycelium which is comparatively uniformly distributed over the surface.

Fig. 2. Strain 13. The mycelium in the centre of the colony is very dense and evenly disposed. There is a narrow band of thin mycelium at the edge.

Fig. 3. Strain 12. The aerial mycelium is very plentiful and is arranged in regular concentric zones.

Fig. 4. Strain 10. The mycelium is very freely produced, has a loose texture and is evenly disposed.

Fig. 5. Strain 21. This strain has a thin and very even young mycelium which is later covered by an irregular thick mycelium which excretes drops of a yellowish brown liquid.

Fig. 6. Strain 18. The freely produced aerial mycelium is arranged in broken concentric zones giving a 'dappled' appearance to the colony.

Fig. 7. Strain 5,7. This is a haploid saltant of strain 5 and has a much thinner mycelium and slower growth-rate than its parent.

Fig. 8. Strain 8. This strain has an evenly produced thin aerial mycelium.

Fig. 9. Strain 5. The aerial mycelium is dense and plentiful in the centre of the colony. A peripheral band about 0.5 cm. wide consisting of very thin hyphae is a characteristic of this strain.

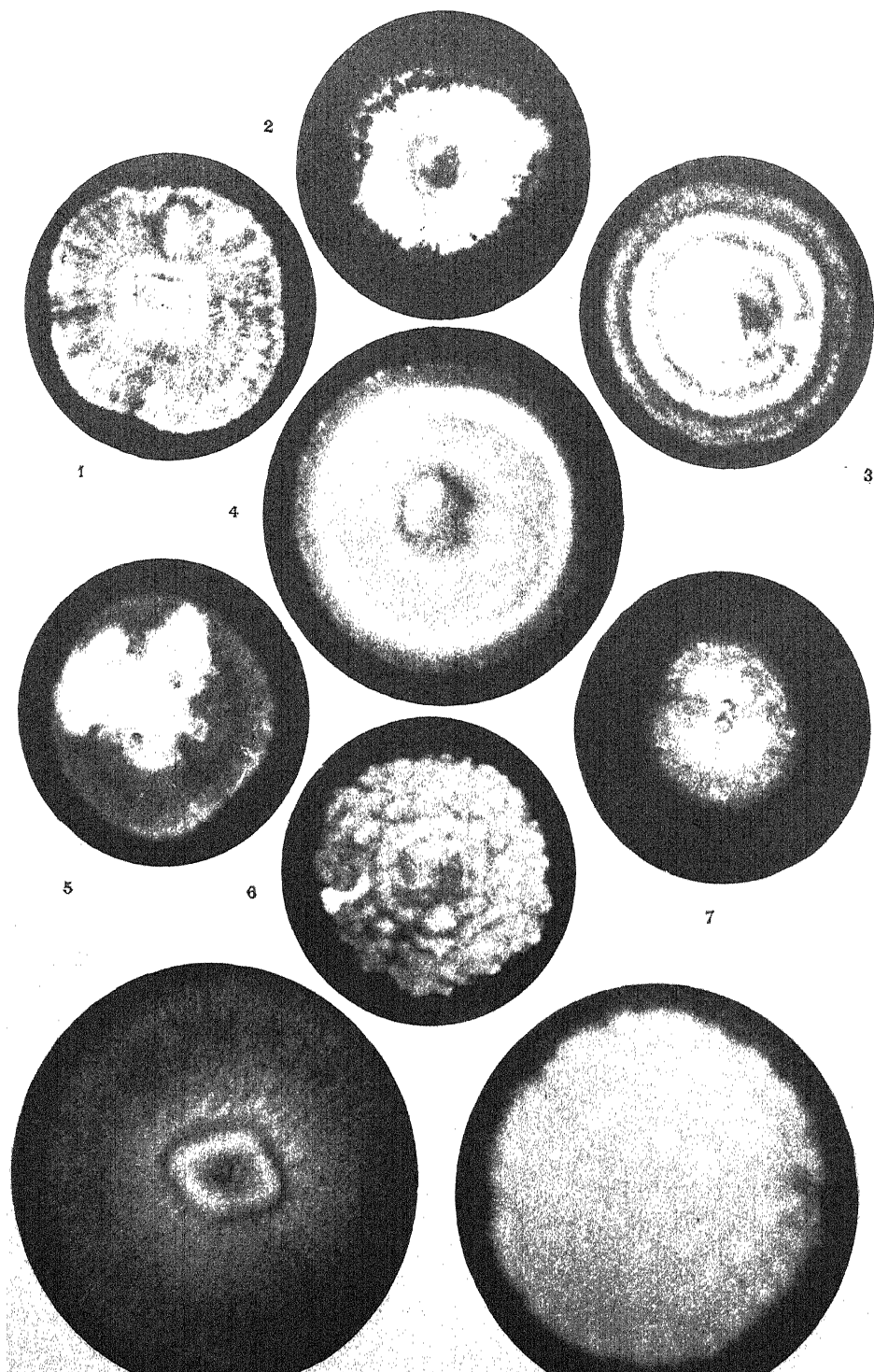
Fig. 10. Strain 12 × 5. This culture has given rise to two perfect fruit-bodies and numerous undeveloped ones can be seen arising generally in the neighbourhood of concentric zones of more plentiful mycelium.

Fig. 11. Strain 8 × 13. This strain is typical of the diploid type which does not give rise to zones of more plentiful mycelium.

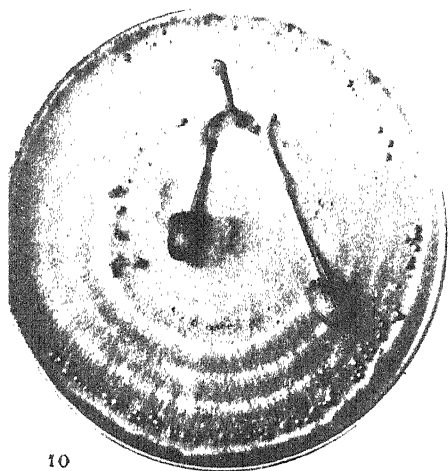
Figs. 12 and 14. These colonies have arisen from inocula taken from an X-rayed culture of strain 5. A saltant sector can be seen in each. The saltants are diploid and have given rise to imperfect fructifications round the edge of the dish. The non-saltated mycelium is much thinner than is that of a colony which has not been irradiated (compare fig. 9).

Fig. 13. Strain 12 × 5,4. This diploid has arisen from strain 12 diploidized by the diploid saltant 5,4. It is very similar to strain 12 × 5, fig. 10.

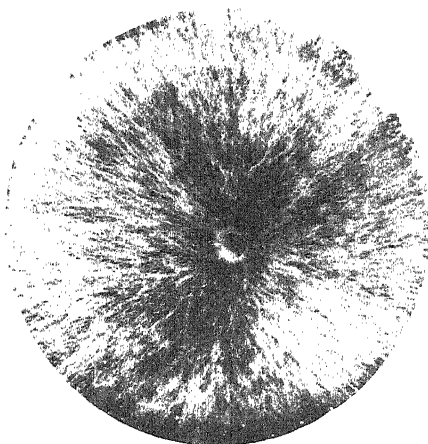
Fig. 15. Strain 5,4. This is a purified culture of the diploid saltant 5,4. Note the imperfect fruit-bodies arising from the single zone of dense mycelium.



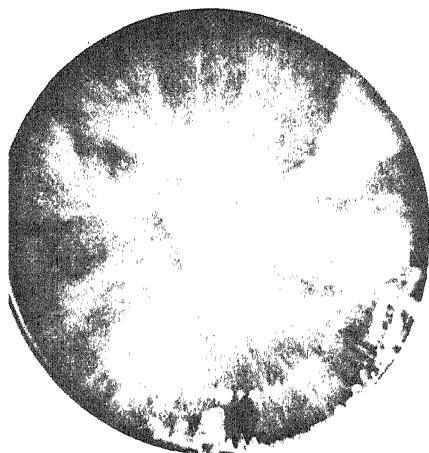




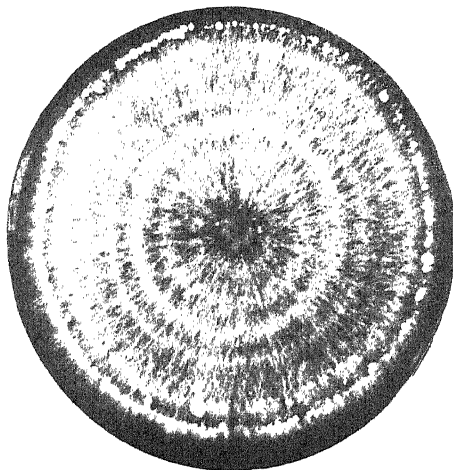
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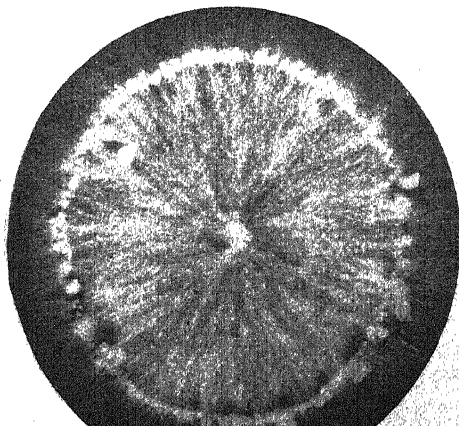
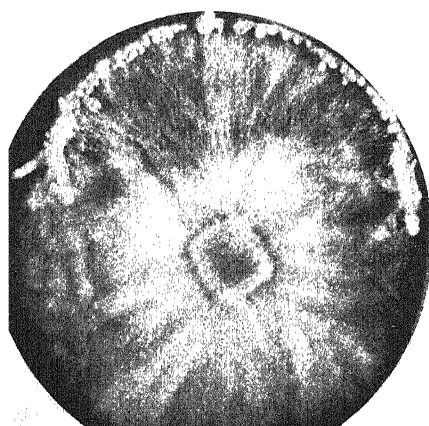
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12



13





# Contributions to the Cytology of *Spermothamnion* *Turneri* (Mert.) Aresch.<sup>1</sup>

## I. The Diploid Generation.

BY

KATHLEEN M. DREW, M.Sc.

(MRS. BAKER).

With Plates XII and XIII and two Figures in the Text.

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### I. INTRODUCTION.

AN outstanding characteristic of the tetraspore-bearing Florideae, which have been investigated cytologically, is a regular alternation of haploid and diploid plants, with the accompanying morphological alternation of plants bearing sexual organs with those bearing tetraspores. In addition, the formation of tetraspores in such species is invariably associated with a reduction division. Svedelius (24) has given the name diplobiontic to this type of life-cycle, and while it seems likely that the majority of the tetraspore-bearing Florideae are diplobiontic, the recent researches on

<sup>1</sup> The material used in this investigation belongs to the species, recognized by American writers as *Spermothamnion Turneri* (Mert.) Aresch. In using this name, the writer does not imply that it is identical with the European material for which the same name or a synonym is used. Other questions as to whether differences between *S. Turneri* (Mert.) Aresch. and *S. roseolum* (Ag.) Pringsh. are of specific rank and as to the claims of the name *S. repens* (Dillw.) K. Rosenv. are left on one side for the time being.

*Phyllophora Brodiaei* (19, 2) and *Gymnogongrus Griffithsiae* (5), show that exceptions exist. In addition, the absence of sexual plants in some species, the preponderance of tetrasporic plants in others, and the occurrence of both tetrasporangia and sexual organs on the same individuals of other Florideae, are facts which suggest that possibly further modifications of this typical life-cycle occur, and in any case they need elucidating.

In view of this, a cytological investigation of *Spermothamnion Turneri* (Mert.), Aresch., as an example of a species in which tetrasporangia and sexual organs commonly develop on the same plant, was undertaken and is the subject of this paper. Although the occurrence of tetrasporangia on sexual plants and conversely of sexual organs on tetrasporic plants has been recorded for a large number of the red algae, in many of these cases it is probably an abnormal event, but the frequency of the occurrence in such cases as *S. Turneri* is enough to justify a consideration of it as normal.

The development of tetrasporangia and sexual organs in close proximity on filaments of both American and European material of *S. Turneri* has been the subject of comment by several writers, including Farlow (4), Lewis (13), Pringsheim (17), Kylin (9), and Rosenvinge (18). More recently, Schussnig and Odle (20) have published an account of the life-history of *Spermothamnion roseolum* (Ag.) Pringsh., which some authorities consider as a growth form of the same species as *S. Turneri*. Like *S. Turneri*, it gives rise to both sexual organs and tetrasporangia on the same plant. The results of this investigation will be referred to subsequently.

## 2. MATERIAL AND METHODS.

The material used in this investigation was collected in the vicinity of Woods Hole, Mass., and the collections were made during the second part of June, through July, and most of August, 1927. A further supply was collected in early August, 1931, by Miss H. T. Croasdale.

*S. Turneri* is a branched filamentous alga and grows epiphytically on other algae, such as *Chondrus*, *Phyllophora*, and *Polysides*. Attached plants are usually found only below low-water level, but quite frequently fragments are torn loose and float in the wash. Much of the material was obtained in this way, in the neighbourhood of Nobska Head, but other plants, growing in forty feet of water, were obtained by dredging between Nobska Head and West Chop on Martha's Vineyard. The plants dredged did not appear to be either so well developed or so richly branched as those found floating, and in some ways they resembled *S. roseolum* (Ag.), Pringsh. Sporangia were not so abundant on this material and sexual organs have not been found. There seemed to be no reason for supposing it to be another species, and the number of chromosomes in a dividing nucleus in a

vegetative cell of the dredged material agreed closely with that of the floating material.<sup>1</sup> The final results have come from a few collections of the floating material, since those plants provided large numbers of nuclei in the process of division.

Fixations were made throughout the day, between 10 a.m. and 1 p.m., and although this usually involved keeping some of the material in the laboratory in sea-water, no damage appears to have resulted.

Material was fixed in twenty-seven standard solutions and modifications of such, but the only one which has given good results is a mixture of 6 c.c. of 40 per cent. formaldehyde and 100 c.c. of 70 per cent. alcohol. Many solutions which have given satisfactory results with the vegetative cells are useless for the sporangia, as in them the middle layer of the wall tends to swell. It swells in some fixatives more than in others, but in all of them the outer layer resists the pressure set up while the inner does not, and so the protoplasm is squeezed into a shapeless mass. The sporangia seem particularly sensitive to fixatives from the end of the early prophase stages of the first nuclear division until the completion of the second division. It has been found necessary to leave the material in the fixative. If transferred to alcohol, it becomes difficult to dehydrate as the filaments tend to twist and collapse.

The filaments have been stained and mounted whole, and in sharpness of nuclear details exceedingly little has been lost by adopting this method. On the other hand, the advantages of working with whole mounts, when it is imperative to know what reproductive organs occur on any given filament, are great.

Brazilin has been employed for staining in order to avoid the use of low percentages of alcohol, in which the sporangium wall swells as in fixatives, other than the one employed. After staining and dehydration in the usual way, the filaments have been cleared by passing them through a long series of alcohol and xylol mixtures. From a final mixture of 1 part of absolute alcohol and 15 parts of xylol, the material has been transferred to a mixture of 3 parts of Canada Balsam, 25 of xylol, and 2 of absolute alcohol. This mixture has then been allowed to concentrate until it reaches a consistency suitable for mounting. Full details of this method will be given later when its use for other algae has been tested.

Owing to the small size of most of the chromosomes and of the other nuclear structures of this plant, special attention has been paid to the apparatus used for making the observations recorded. The use of a Watson Holoscopic Immersion Condenser has been particularly helpful. Critical illumination, with a Pointolite lamp as the source of illumination, has been employed throughout the investigation.

<sup>1</sup> This supports Rosenvinge's (18) opinion that *S. Turneri* (Mert.) Aresch. and *S. roseolum* (Ag.) Pringsh. are but different forms of the one species.

## 3. CYTOLOGY OF DIPLOID PLANTS.

As several plants probably combine to form the tufts, which are so characteristic of the habit of growth of *S. Turneri*, it is not easy to define the exact limits of a single plant. There is no question, however, that, as other writers (17, 4, 13, 9, and 18) have described, tetrasporangia occur on the same filaments as procarpic and antheridial branches and cystocarps (Text-fig. 1, A and B). They may occur in close proximity, and tetrasporangia are formed quite commonly on branchlets terminated by a procarp and also from the cell, from which the involucrel filaments of the developing cystocarp usually arise. In one case a sporangium (*s*) was found to be developing from a cell of the procarp itself (Text-fig. 2, B).

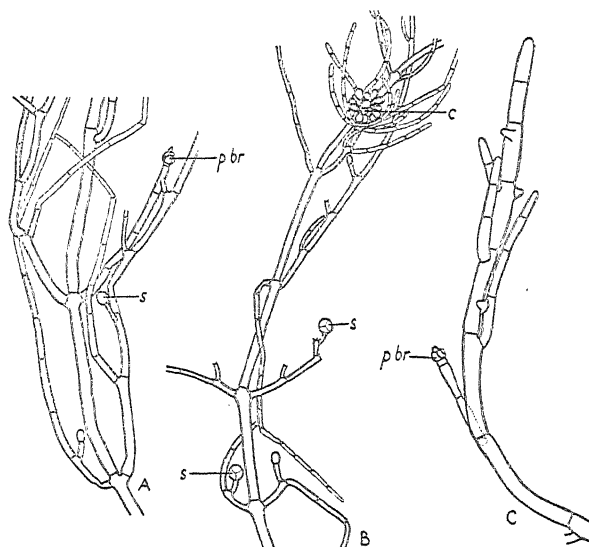
So far, the only adult filaments found with their nuclei in the process of division belong to diploid plants, the number of chromosomes in the nucleus being sixty. As might be expected, tetrasporangia are found on such filaments, but procarpic branches and cystocarps occur as well. Antheridial branches are rare in the material, and in only a single case has one been found on a filament known to be diploid. This antheridial branch appeared to be mature and healthy. Although the tetraspores germinate readily, no adult haploid filaments have come under notice. It has still to be ascertained therefore, whether like the diploid, they bear both sexual and asexual reproductive organs.

Although procarpic branches occur in varying numbers on most of the material examined, tetrasporangia are by far the more numerous. Mature cystocarps are found usually as isolated examples, but developing cystocarps are fairly numerous in some of the June material. Antheridial branches occur very rarely on filaments fixed in late June and early August, but some of them do not look very healthy. This is the first record of antheridia of *S. Turneri* on the American Coast.

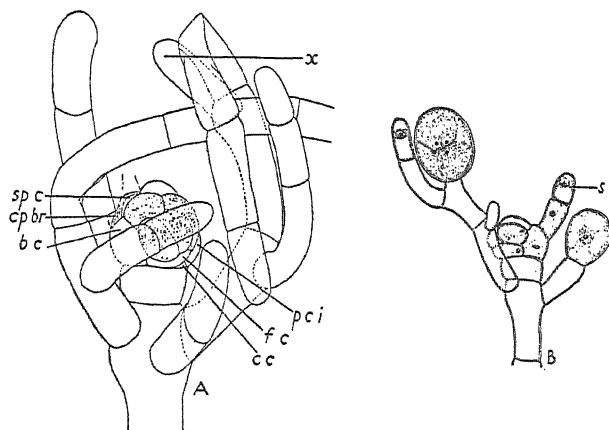
In the following pages, detailed descriptions of the division of the somatic nuclei, the nuclear history of the tetrasporangium, and the development of, and nuclear divisions in, developing procarpic branches and cystocarps of diploid plants are given. Following these, the germinating tetraspores are described and the bearings of these observations on a conception of the life-history of *S. Turneri* are discussed.

(a) *Somatic Nuclear Divisions.*

Cells other than those concerned with reproduction are multinucleate. The germinating spore quickly changes from the uni- to the multi-nucleate condition (Pl. XIII, Fig. 37), and some adult cells may contain as many as thirty nuclei. These all divide at the same time, but the nuclei in the apical end of the cell are usually slightly in advance of those at the basal end. This is shown very clearly by the cell represented in Pl. XII, Fig. 9,



TEXT-FIG. 1. A Filament bearing procarpic branch (*p br*) of Pl. XIII, Fig. 36, and young sporangium (*s*), the nucleus of which is figured on Pl. XIII, Fig. 38.  $\times 59$ . B Portion of plant bearing both cystocarp (*c*) and mature tetrasporangia (*s*).  $\times 32$ . C Filament, the apical cell of which is figured on Pl. XIII, Fig. 37. Procarpic branch (*p br*) of Pl. XIII, Fig. 33 borne on this filament.  $\times 83$ .



TEXT-FIG. 2. A Developing cystocarp with involucre filaments. Diploid nucleus of apical cell marked *x* and tetraploid nucleus of cell of gonimoblast on back side of procarp represented on Pl. XIII, Figs. 42 a and 42 b respectively. Cells of upper gonimoblast dotted. *sp c* = sporogenous cell; *cp br* = remains of carpogonial branch; *bc* = basal cell; *p c 1* = first pericentral cell; *fc* = foot cell; *cc* = central cell.  $\times 333$ . B Filament bearing abnormal procarpic branch, from a cell of which a sporangium (*s*) is developing. The cell immediately below the procarp has also given rise to sporangia-bearing branches.  $\times 205$ .

the basal nuclei of which are at metaphase, whereas the apical ones are at anaphase and telophase.

In interphase the nuclei of the apical cells are spherical, but those of

older cells are much flattened and lie closely against the walls, in the thin layer of cytoplasm. The nucleus has a prominent nucleolus (Pl. XII, Fig. 1) except in older cells which have ceased to divide. Surrounding the nucleolus is the karyotin, which seems to vary in amount, appearance, and capacity for taking up brazilin, even in corresponding cells. In rapidly dividing apical cells it most frequently has the form of irregular branched strands (Pl. XII, Fig. 1) and as the time of division approaches these thicken and segment to give the chromosomes. The chromosomes are not easily distinguishable at first, but gradually become more and more distinct, more definite in outline and larger. At this stage they are scattered throughout the nuclear area, around the nucleolus (Pl. XII, Fig. 2, and Pl. XIII, Figs. 37 and 37 a). Except in older cells, where the nucleus is much flattened, the chromosomes can then be counted comparatively easily. The number distinguishable is either sixty (Pl. XII, Fig. 2) or slightly less (Pl. XIII, Fig. 37 a), and corresponds with the thirty bivalent chromosomes found in the tetrasporangium at diakinesis. Although there are sixty chromosomes in the nucleus represented in Pl. XII, Fig. 2, only fifty-six are visible in the figure as the remaining four are covered by others. In the nucleus of Pl. XIII, Fig. 37 a the chromosomes total fifty-eight, and two of these are completely hidden beneath others.

After the chromosomes have been formed the nucleolus soon fades away and the chromosomes move towards the equatorial plate, some lagging behind the others (Pl. XII, Fig. 3). They become smaller as metaphase approaches and some appear to be slightly longer than broad at this stage (Pl. XII, Fig. 3), but the angle of view probably determines the shape. When on the plate it is not possible to see them all individually, due possibly to crowding or to the unsuitability of the fixative for this stage (Pl. XII, Figs. 4 and 5). By metaphase the nuclear membrane has become so thin that it is not visible as such, but there is evidently some boundary as an area of clear protoplasm, elliptical in section, surrounds the plate and contrasts with the rest of the protoplasm which is much more granular (Pl. XII, Figs. 4, 5, and 6). Sometimes this area is not well defined, especially in older cells, and it always becomes less marked as the division proceeds. Even at telophase, however, the protoplasm around the chromosomes is slightly clearer than the rest (Pl. XII, Figs. 7 and 9). There seems to be nothing to determine the direction of the spindle (Pl. XII, Fig. 9) and neither centrosomes nor spindle fibres have been seen during nuclear divisions. As the two groups of chromosomes move apart, after division, a few faint, thread-like connexions remain between some of them (Pl. XII, Figs. 6 and 9). After metaphase it is no longer possible to estimate the number of chromosomes present in each group, as they appear to be more or less fused together to form a single mass. Sometimes spherical bodies can be distinguished in these masses moving towards the



poles (Pl. XII, Figs. 6 and 9), but it is probable that these are not single chromosomes. The masses become more homogeneous as they approach the poles, where their shape changes from that of a flat plate to that of a shallow basin, with its concave side towards the pole (Pl. XII, Fig. 7). A thin membrane then arises on the polar side of each mass and completes a sphere within which the nucleus reorganizes. At an early stage, the mass of karyotin becomes more irregular in shape and gives rise to the thin thread or threads of interphase (Pl. XII, Fig. 8). What remains after the thread has been formed starts to take the stain more readily and rounds up as the nucleolus.

(b) *The Development of, and Nuclear Divisions in, the Tetrasporangium.*

Tetrasporangia arise both terminally and laterally on short branches of the main filaments. These short branches may be branched themselves, in which case, sporangia, in all stages of development, are clustered together. The tetrasporangium-mother-cell is always uninucleate so that it is easy to distinguish an apical cell from which a sporangium is to be derived, by the presence of a single isolated nucleus in the upper half (Pl. XII, Fig. 10). This nucleus is unusually large and is soon cut off by a wall formed in the usual way, i.e. by ingrowth from the periphery.

Although the young sporangium so formed increases in size, its proportions remain much the same until the later prophase stages, when it starts to become more spherical, by an increase in the transverse diameter. The process of rounding up is hardly complete by the end of the second nuclear division.

After the sporangium has been cut off from the stalk-cell, the nucleus rapidly increases in size and continues to do so until the metaphase of the first division is approaching (Pl. XII, Figs. 11-18). It has a prominent nucleolus, and around this is the slender spireme pursuing an irregular course throughout the nuclear area (Pl. XII, Fig. 11). At this stage it is impossible to be certain whether the spireme is a continuous thread or not. During prophase it undergoes many changes and appears in very different forms at each succeeding stage.<sup>1</sup> As these changes occur in sporangia borne on the same filament as sporangia with their nuclei at diakinesis, they undoubtedly represent rearrangements which are a necessary precedent to reduction division. Very soon after the dividing wall has been completed, the nucleus takes on an appearance, resembling that described for synzesis in other plants. The spireme thread becomes densely coiled and occupies only a third or a quarter of the nuclear area (Pl. XII, Fig. 12). It has not been possible to find any direct evidence that syndesis occurs during this period of contraction, as the mass is very tangled and the thread very fine.

<sup>1</sup> Owing to the great complexity of detail in the nuclei illustrating the early stages and shown on Pl. XII, Figs. 11-14, it has been impossible to reach the same accuracy of representation as in the case of the other figures.

There is no doubt, however, that towards the end of this stage, the spireme is shorter and thicker. When it spreads throughout the nuclear area once more, loops of twisted thread such as those represented in Pl. XII, Fig. 12 a, are seen. Whether these loops have any significance or are accidental cannot be determined. After synizesis the nucleus enlarges, but chiefly laterally, thus becoming oval in section. The nucleolus enlarges also and the spireme is now considerably thicker than before the contraction. Its outline is very even, and instead of pursuing a very meandering course, as originally, it traverses the nuclear area in long curves. This stage is a very distinctive one, and judging from the number of sporangia found with their nuclei in this condition, it must be of considerable duration. There is evidence that during this time the spireme segments into definite lengths for, whereas in the nuclei of smaller sporangia the spireme seems more or less continuous, in the nuclei of larger sporangia there are several distinctly separate threads as in the nucleus of Pl. XII, Fig. 13. Since these do not number more than thirty (the haploid chromosome number), it seems safe to assume that each piece represents a bivalent chromosome. Intermediate stages are represented by the nuclei in which there are both long and short lengths. The longer pieces are often knee-jointed, suggesting that they are about to segment at the bend. The nucleus gradually passes from this stage into one in which the bivalent chromosomes are more distinctly separate from one another and their double nature is apparent by diamond-shaped openings and forked ends (Pl. XII, Fig. 14). They are no longer of even thickness and fairly straight, but knotted and twisted. The nucleus and nucleolus continue to enlarge, and the former becomes spherical again (Pl. XII, Fig. 15). At the same time the bivalent chromosomes shorten, but the members of each pair tend to separate further from one another. They appear in various forms of which the most common is a diamond-shaped ring with accumulations of material at the corners, especially two opposite ones (Pl. XII, Figs. 15 and 16). The chromosomes lose, for a time, their affinity for brazilin but regain it as they shorten and thicken for diakinesis. Several stages in this condensation for diakinesis are to be found in the same nucleus, as will be seen from Pl. XII, Fig. 28. During diakinesis each chromosome of the pair is more or less spherical, and although lying closely against its neighbour, is quite separate from it (Pl. XII, Fig. 17). At the same time, some of the univalent chromosomes ( $x$ ) show indications of the beginning of the split which takes place in the homotypic division (Pl. XII, Fig. 17).

The occurrence of such a characteristic diakinesis shows that pairing of the chromosomes has occurred during the events which have been described. The actual process of pairing has not been observed, but judging from the details recorded, it seems likely that it takes place during or immediately after synizesis. Additional evidence that the first nuclear

division in the sporangium is a reduction division, is provided by the two nuclei of Pl. XII, Figs. 29 and 28. Both nuclei are from the same filament, the former being that of a stalk-cell of a sporangium and the latter the nucleus of a tetrasporangium-mother-cell. Whereas the vegetative nucleus is in prophase and at least fifty-five chromosomes are distinguishable (one of these being hidden in the figure), the nucleus of the tetrasporangium-mother-cell is nearing diakinesis and appears to have thirty-two bivalent chromosomes. The usual number in a sporangium nucleus at this stage is thirty, but it is not always easy to distinguish between chromosomes and deeply-staining bodies lying immediately outside the nucleus, and that probably accounts for the high number in this case.

When the bivalent chromosomes in the nucleus of the tetrasporangium are fairly well condensed, the nucleolus loses its affinity for stains and sometimes becomes vacuolate, but in all cases eventually disintegrates. In the last stages of disintegration it may take on quite an irregular shape (Pl. XII, Fig. 18) (cf. *Corallina officinalis* var. *mediterranea* (3)). A very thin nuclear membrane persists, for the nuclear area is still quite well defined (Pl. XII, Fig. 18). During the separation of the chromosome pairs, however, the nuclear area gets gradually smaller (Pl. XII, Figs. 18-20) and whereas until metaphase the substance filling the nuclear cavity is homogeneous in appearance, it then becomes granular (cf. Pl. XII, Figs. 19 and 20). During late anaphase and early telophase the nuclear membrane vanishes, at first in the equatorial region and then progressively towards the poles (Pl. XII, Fig. 21).

No indication of an achromatic figure has been seen, but just prior to metaphase denser masses of protoplasm appear at the poles pressed against the outside of the nuclear membrane and such as are indicated in the later stage of Pl. XII, Fig. 20, (*p*). During anaphase gradually increasing areas of clear protoplasm appear in the centres of these masses (*p*) (Pl. XII, Fig. 21). Eventually the karyotin of telophase enters these (Pl. XII, Fig. 22) and the daughter nuclei are reorganized in them (Pl. XII, Fig. 22).

After diakinesis the two members of each of the thirty bivalent chromosomes fuse together to form a single body (Pl. XII, Fig. 18), and when the nucleolus has completely disappeared they move to the equatorial plate (Pl. XII, Fig. 19). In the first nuclear division this is always at right angles to the long axis of the sporangium. Some of the chromosomes lag behind throughout the division; thus in Pl. XII, Fig. 19, while some are on the plate and are undergoing disjunction others are quite far off. It will be noticed also, that disjunction may occur either before or after the plate is reached (Pl. XII, Fig. 19). Pl. XII, Fig. 20, shows most of the bivalent chromosomes arranged on the plate, many of them in disjunction, the chromosomes of one pair (*a*) having completely separated. As these bivalent chromosomes separate they are dumb-bell shaped, the two

members of a pair often remaining connected by a very thin fibre, even after they have moved a considerable distance apart (Pl. XII, Figs. 20 and 21). The chromosomes retain their individuality until late anaphase (Pl. XII, Fig. 21), but in telophase they fuse together to give a structureless mass which is often saucer-shaped, the concave side being towards the pole. This becomes adpressed to the polar area of clear protoplasm, enters it (Pl. XII, Fig. 22) and there gives rise to both the nucleolus and the karyotin of the daughter nucleus (Pl. XII, Fig. 23). The daughter nuclei are completely reorganized between the first and second division and probably spend a long period in interphase (Pl. XII, Fig. 24), judging from the number of binucleate sporangia found. During interphase the nucleolus is prominent and shows great affinity for brazilin in contrast to the spireme. As prophase approaches the spireme takes the stain more readily again and the chromosomes are formed very quickly. They number thirty. The nuclear area is smaller than that of the first nuclear division and the details are not so easy to follow. Prophases of the two sister nuclei of the second nuclear division are represented in Pl. XII, Fig. 25, which also shows the smaller size of the chromosomes. They number thirty in both nuclei. The nucleolus vanishes as in the first division and the chromosomes move to the plate (Pl. XII, Fig. 26). The two equatorial plates are usually in planes at right angles to one another, so that one plate is seen in surface view and the other in side view (Pl. XII, Fig. 26), or else both are seen obliquely. The metaphase of the second division is very much more like that of a somatic mitosis than that of the first nuclear division of the sporangium, in that the chromosomes are much closer together and the nuclear area is not so well defined. During anaphase the chromosomes are not distinguishable individually, and before telophase they have fused into a structureless mass as in Pl. XII, Fig. 27, which shows the products of the division of one nucleus only. From this, both the karyotin thread and the nucleolus of the daughter nucleus are formed. Although the material examined contains large numbers of mature sporangia and many showing all stages of the first division, very few examples of the second division have been found, suggesting that it is passed through more rapidly than the first. The four daughter nuclei are completely reorganized at the periphery of the sporangium and then move back to the centre, where they become arranged tetrahedrally.

After the nuclear divisions are completed there is a period of growth during which the sporangium attains its full size. Invagination of the cytoplasm which starts during this period of growth, never begins until the four nuclei have reached the centre of the sporangium, in contrast to *Delesseria sanguinea* (23). An early sign of invagination in living material is the appearance of two furrows, each approximately a third of the circumference of the sporangium in length, on opposite sides of the

sporangium and at right angles to one another. Owing to the transparency of the protoplasm, this gives rise to a well-formed  $\times$ , if viewed from the appropriate angle. It is not certain whether the four further furrows, which connect the ends of the original two, form at the same time or later. They all deepen, and when the process is about two-thirds complete, there is a pause before the spores become entirely separated, as in *Griffithsia Bornetiana* (13).

Since no purely sexual or purely tetrasporic plants have been found, it seems reasonable to expect that some tetraspores might be formed on haploid plants without a reduction division. So far no evidence of this has been obtained, since no known adult haploid individuals have been found and all the sporangia investigated develop in the same way.

Occasionally sporangia containing more than four spores are found, a fact which may be correlated with the occurrence of what seem to be binucleate sporangium initials. These binucleate initials are quite distinct from sporangia in the binucleate stage following a division.

In addition, three sporangia with multinucleate spores (Pl. XII, Fig. 30) have been seen and must probably be considered as abnormalities. None of these sporangia are borne on filaments whose chromosome complement is known.

(c) *The Development of Procarpic Branches and Cystocarps on Diploid Plants.*

The structure of the procarpic branch in the genus *Spermothamnion* has been investigated by several of the earlier workers on the Algae (15, 17, 1, 6) and more recently by Kylin (10), and Schussnig and Odle (20). The nuclear history of the developing procarpic branch, on the other hand, has received no attention apart from brief mention by the last-mentioned authors and Rosenvinge (18), nor have the nuclear phenomena, which initiate the development of the gonimoblasts been followed. These details, which are of particular interest in the case of procarpic branches formed on diploid plants, will now be described for *S. Turneri*.

The earliest stage seen is that of a small binucleate cell terminating a short branch. Whether such a cell is uninucleate originally is unknown. The two nuclei divide to give four, but although this suggests that a reduction division may take place, this cannot occur as the diploid number of chromosomes is found in all the subsequent divisions. A transverse wall is now laid down and three of the nuclei are cut off by it, from the fourth, which remains in the apical cell of the branch. Such a procarpic branch is shown on Pl. XIII, Fig. 31. The single nucleus of the apical cell enlarges and divides, and the nucleus (Pl. XIII, Fig. 31a) of the procarpic branch referred to is in the prophase of this division. There appear to be fifty-nine

chromosomes in this nucleus (three of which are not visible in the figure). After this nuclear division another wall is laid down parallel to the last, so that the procarpic branch then consists of a row of three cells of which the middle one is the largest. The apical and middle cells are uninucleate, but the basal one contains three nuclei. The apical and basal cells do not divide further, nor do they enlarge as much as the central cell, from which three pericentral cells are then cut off in succession. The first of these three pericentral cells to be formed is a small one on the abaxial side of the branch (Pl. XIII, Fig. 32, *p.c.* 1). The second, however, is much larger (Pl. XIII, Fig. 32, *p.c.* 2) and soon cuts off a small apical portion (Pl. XIII, Fig. 33, *st.c.*) known as the sterile cell. The nucleus (Pl. XIII, Fig. 32 a) of the second pericentral cell of the procarpic branch represented in Pl. XIII, Fig. 32, is in the prophase of the division which precedes the formation of this cell, and the diploid number of chromosomes is present (three being completely hidden in the figure). After the division of the second pericentral cell a third is cut off. The nucleus of the central cell (Pl. XIII, Fig. 33 a) of the procarpic branch of Pl. XIII, Fig. 33, is in prophase prior to the formation of this cell, and here again the chromosomes number sixty (four of which are not visible in the figure). This procarpic branch is also of interest since it is borne on the same filament (Text-fig. 1 C), the apical cell of which is represented on Pl. XIII, Fig. 37. The nuclei of this cell are also in prophase, and there are fifty-eight chromosomes in the nucleus figured in detail (Pl. XIII, Fig. 37 a). This filament therefore, provides undoubted evidence that no reduction division precedes the formation of procarpic branches on diploid plants.

The first of the three pericentral cells does not divide again, and the third remains undivided until after the fertilization of the carpogonium. The basal of the two cells, formed from the second pericentral cell, gives rise laterally to the mother-cell of the carpogonial branch, and so is the supporting-cell (*tragzelle*) (Pl. XIII, Fig. 33, *s.c.*). The mother-cell of the carpogonial branch divides into four cells, which are so shaped that the branch is curved in three directions and comes to occupy an adaxial position between the second and third pericentral cells. The apical cell of the branch forms the carpogonium.

The three pericentral cells of the procarpic branch are all uninucleate as is also the sterile cell, which is cut off from the supporting-cell. In addition, the cells of the carpogonial branch are all uninucleate at first but usually become binucleate later (Pl. XIII, Fig. 35, *cp. br.* 2). The carpogonium is uninucleate when formed, but the nucleus subsequently divides, one of the daughter nuclei passing into the trichogyne.

It has not been possible to follow all these nuclear divisions during the development of the carpogonial branch, but wherever possible counts have been made of the number of chromosomes appearing in the nuclear

divisions in any of these cells. In every case it is sixty or approximately so, and some of these nuclei are represented in Pl. XIII, Figs. 34 and 34 a, 35 and 35 a, 36 and 36 a. In Pl. XIII, Fig. 34, it is the nucleus of the apical cell of a three-celled carpogonial branch which is in prophase, and sixty chromosomes (three of which are hidden in the figure) are distinguishable (Pl. XIII, Fig. 34 a). It is interesting to note that the trichogyne has started to develop, although the last cell-division has still to take place. In another example, fifty-seven chromosomes (one of which is under the nucleolus) appear in the nucleus (Pl. XIII, Fig. 35 a) of the third cell of a carpogonial branch (Pl. XIII, Fig. 35, *cp. br.* 3). In the procarpic branch of Pl. XIII, Fig 36, the nucleus of the carpogonium itself is in prophase, and the division will result in the formation of the egg and trichogyne nuclei. The nucleus (Pl. XIII, Fig. 36 a) is diploid, as fifty-nine chromosomes are distinguishable (two of which are hidden beneath others in the figure). The nucleus of a tetrasporangium (*s*), borne on the same filament (Text-fig. 1, A) as this procarpic branch (*p. br.*) is reproduced on Pl. XIII, Fig. 38. It is approaching diakinesis and contains thirty bivalent chromosomes, showing that the diploid number of chromosomes in the carpogonium is due to its formation on a diploid plant and not to a preceding nuclear fusion.

There is no difficulty, therefore, in showing that diploid procarpic branches are initiated and mature. Moreover, the diploid nature of the procarpic branch does not inhibit further development, for although there is a high mortality at this stage, some of these diploid carpogonia develop into cystocarps. Evidence of this is provided by the occurrence of cystocarps on plants known to be diploid on account of the number of chromosomes present in the nuclei of either vegetative cells or cells of the involuclal filaments. A particularly interesting example of this is shown in Text-fig. 2, A. The nuclei of one of the apical cells of the rapidly dividing involuclal filaments are in prophase ( $\times$ ) and the nucleus of one of the cells of the developing gonimoblasts is in the same condition. Whereas the chromosomes in the somatic nucleus (Pl. XIII, Fig. 42 a) number fifty-nine or sixty (three of which are not visible in the figure, however), the nucleus of the gonimoblast has one hundred and ten (Pl. XIII, Fig. 42 b). Ten of the latter are not shown in the figure as they occur beneath others. This is therefore a diploid plant bearing a tetraploid cystocarp, and it shows that although no nuclear fusion has been seen the formation of the cystocarp is initiated by such an event.

The question whether this tetraploid condition is the result of diploid carpogonia being fertilized in the normal manner is obviously the first to be asked. Since antheridial branches occur so rarely, this might well be doubted. Once further development of the carpogonium has started, the trichogyne seldom remains intact, but in cases where it does spermatium-

like bodies are sometimes seen adhering to it. In all probability, therefore, fertilization does occur. The procarpic branch of Pl. XIII, Fig. 39, moreover, shows that the nuclear fusion takes place in the carpogonium (*cp.*) and so is probably the normal one, as it contains two tetraploid nuclei, the plant being diploid.

In addition, the procarpic branch referred to shows some of the first developments which take place in connexion with the formation of the cystocarp. Changes in the cell contents of the carpogonial branch have started, the first nuclear division in the carpogonium has already taken place and both auxiliary cells have been formed. The two nuclei of the carpogonium are in the prophase of the second division. One of these nuclei is reproduced in detail (Pl. XIII, Fig. 39 a) and the chromosomes number one hundred and eleven. Although the chromosomes have been drawn rather small to prevent much overlapping, eleven are hidden completely in the figure. The two nuclei of the central cell of the procarpic branch and also the nucleus of each basal cell are also in prophase, the number of chromosomes present showing the plant to be diploid. To avoid confusion, the only one of these four nuclei shown in Pl. XIII, Fig. 39, is that of the basal cell (*b.c.*) of the auxiliary (*a.c.* 1) formed from the supporting-cell. It appears in the centre of the back side of the procarp. This nucleus, which is given in greater detail in Pl. XIII, Fig. 39 b, has fifty-six or fifty-eight chromosomes (two of which are not visible in the figure).

Nuclear divisions in the carpogonium cease with the formation of four nuclei. Two of these pass into the two small sporogenous cells, which are formed, one on each side of the carpogonium, near the auxiliary cells. The remaining two nuclei do not leave the carpogonium but gradually disintegrate and, like the nuclei of the other cells of the carpogonial branch, their remains can be distinguished when the gonimoblasts are well developed. Occasionally only one sporogenous cell is formed and consequently but one gonimoblast results.

While the nuclear divisions in the carpogonium are taking place the supporting-cell and third pericentral cell enlarge. Each cell has a single nucleus which also increases in size, and, although the karyotin does not take up the stain at all readily, the nucleolus does. Both of these cells then divide into an upper large cell, the auxiliary cell (Pl. XIII, Fig. 39, *a.c.* 1) and a lower smaller cell, the basal cell (Pl. XIII, Fig. 39, *b.c.*), which is in direct connexion with the central cell of the procarpic branch. Each basal cell is uninucleate at first, but soon becomes binucleate, the prophase of such a division being reproduced in Pl. XIII, Fig. 39 b. Each auxiliary cell is uninucleate at first, and, although the nucleus is large and has a prominent nucleolus, there is no indication of a karyotin thread, the nuclear area being clear (Pl. XIII, Fig. 39, *a.c.* 1). Each auxiliary cell enlarges



considerably after its formation, and the nucleus moves to the basal and outer side of the cell, where it divides. Both daughter nuclei are cut off with a small amount of protoplasm to form the foot cell (Pl. XIII, Fig. 40, *f.c.*), the remaining portion forming the central cell from which a gonimoblast develops (Pl. XIII, Fig. 40, *g.c.*). In procarpic branches, such as that of Pl. XIII, Fig. 40, showing the foot cell about to be cut off, the sporogenous cell (*sp.c.*) on that side of the procarp seems to be in open connexion with the neighbouring central cell (*g.c.*). Each sporogenous cell is uninucleate, and in the upper end of each central cell there is a nucleus with faintly staining karyotin and two or more small nucleoli. In all cases the central cell nucleus is quite unlike the two nuclei of the related foot cell, but closely resembles the nucleus of the adjacent sporogenous cell. It seems therefore that, although the division has not been seen, the nucleus in the central cell and the one in the communicating sporogenous cell are daughter nuclei of the original sporogenous cell nucleus. This being so, the nucleus of the central cell is derived therefore from the carpogonium. The multinucleolate condition of this nucleus is worthy of note. 'Where there are just two nucleoli the appearance might suggest a nuclear fusion, but as there are sometimes more than two, and as the sporogenous cell nucleus is similar, this explanation will not suit. It seems more likely that the nucleolus fragments, in order to move more easily from the sporogenous cell to the central cell, although the sister nucleus remains in situ, it is evidently influenced by the same stimulus. The latter, like the nuclei of the foot cell, shrivels and takes no part in the development of the gonimoblast. The gonimoblasts, one on either side of the procarp, result from the divisions of the central cells and their nuclei derived from the carpogonium. At first the divisions are fairly orderly, but before long a very irregular mass results. The outermost cells become carpospores simply by a change in the shape of the outer wall, the resulting spores being pear-shaped.

Occasionally nuclei of the developing gonimoblasts are found dividing, and two in prophase have been found with one hundred and ten and one hundred and twelve to one hundred and fifteen chromosomes respectively, indicating a tetraploid nature. The former nucleus is reproduced in Pl. XIII, Fig. 42 b, but the chromosomes being so numerous, ten are not represented in the figure.

That triploid carpospores may occur as well as tetraploid is suggested by the nucleus in a cell of another gonimoblast. The nucleus has eighty or eighty-two chromosomes, only seventy of which are shown in the figure (Pl. XIII, Fig. 41). It is interesting to note that carpospores are maturing in this cystocarp. This triploid nucleus supports the view that the carpogonia are fertilized by spermatial nuclei in the normal manner, for if both diploid and haploid spermatia occur, then diploid, triploid, and tetraploid carpospores would result. If, on the other hand, normal fertilization is

replaced by another nuclear fusion only diploid and tetraploid carpospores might be expected.

Pl. XIII, Fig. 43, is interesting in this connexion, as it shows a haploid nucleus ( $n$ ) in the trichogyne of the carpogonium. The nucleus is represented in greater detail in Pl. XIII, Fig. 43 a. The egg-cell, whether fertilized or not, is already cut off from the trichogyne. Possibly the plant itself is haploid, but in addition to the nucleus in prophase, there is another in a position suggesting movement down the trichogyne, and what seems to be the remains of a third near the base. It looks, therefore, as though they are male nuclei, but at the same time there are no signs of attached spermatia on the trichogyne unless they are adhering immediately above or below, in which positions it might not be possible to distinguish them.

Other changes take place in the procarpic branch during the formation of the cystocarp. The nuclei of the basal cell of the procarpic branch divide as the cell enlarges, so that eventually it may contain as many as thirteen nuclei. The nuclei of the central cell divide once or twice and the nucleus of each of the three cells, the apical, the first pericentral, and the sterile, divides once. The protoplasm in the basal and central cells of the procarpic branch becomes denser, but the other cells not directly concerned with the developing cystocarp gradually lose their protoplasm.

The cells of the carpogonial branch do not divide after fertilization, but probably supply nourishment to the carpogonium until the nuclear migration to the auxiliary cells has taken place, for although at first they are full of dense protoplasm it rapidly disappears. While this is happening the nuclei of these cells lose their nucleoli, enlarge, and take on the appearance, characteristic of nuclei of cells, invaded by parasites (Pl. XIII, Figs. 39 and 40). After the cells have lost their protoplasm, however, the nuclei dwindle in size, but can be distinguished some time after the gonimoblasts are initiated. In some cases the carpogonial branch shrivels and remains as a dark mass, the nuclei being indistinguishable.

Involucral filaments, which are branches of the cell immediately below the procarp, grow up round the developing gonimoblasts and probably provide a certain amount of protection.

Several abnormalities have been found during this investigation, and some are worthy of note. Of these, the most commonly occurring are carpogonial branches with multinucleate (Pl. XIII, Fig. 44) or multicellular (Pl. XIII, Fig. 45) trichogynes. In addition, five procarps with spore-like outgrowths of the carpogonial branch have been found amongst the material examined. The outgrowths closely resemble carpospores both in shape, size, and appearance. Although it has not been possible to determine its origin exactly, in all cases the spore must have come from either the third cell of the carpogonial branch or the carpogonium itself. Another frequent abnormality is the division of the carpogonium to give a cell

mass. These carpogonia are probably unfertilized, as there are no spermatia on their trichogynes and the auxiliary cells have not been formed. The absence of one sporogenous cell, with the corresponding sterility of the adjacent auxiliary cell has been referred to already.

#### GERMINATING TETRASPORES AND THE HAPLOID GENERATION.

The early stages in the germination of the tetraspores have been followed. This can be done quite easily by placing slides at the bottom of a bowl of sea-water, in which plants bearing tetrasporangia are suspended, late at night. Many spores are liberated and adhere to the slides, on which they can then be fixed and stained. The tetraspores germinate very rapidly, and within twenty-four hours of liberation may become three-celled, each cell containing two or three nuclei. After this, growth in the laboratory becomes extremely slow, probably due to unfavourable conditions. Although much to be desired, no cultures under natural conditions have been made.

The first nuclear division in the germinating spore has not been seen, as this evidently occurs before the spore attaches itself to the substratum. In all subsequent divisions thirty chromosomes, or approximately that number, appear in the prophase of the nuclear divisions. This is so in the case of the nucleus of the germinating tetraspore of Pl. XIII, Fig. 46, but in the detailed figure (Pl. XIII, Fig. 46 a) one chromosome is beneath the nucleolus. As thirty is the number of the chromosomes found in the anaphase of the first and the prophase of the second nuclear divisions in the tetrasporangium on diploid plants, there is no reason to suppose that the first division in the germinating spore differs in any way from any other somatic division.

So far, these sporelings provide practically all the evidence of a haploid generation, as all the mature plants for which the chromosome number is known, are diploid. The absence of adult plants known to be haploid may be due to one of two causes. In the first place the nuclei of the haploid plants collected may not be in division, or else *Spermothamnion Turneri* is possibly like *Ceramium corticulatum* (25) and other Florideae in having a seasonal alternation of generations. The collections used for this investigation were made over a period only just exceeding two months, and the material which has given the final results covers an even shorter period of time. There is no reason to suppose, therefore, that these sporelings described never develop into mature plants, and that further searching will not reveal adult plants with the haploid chromosome number. As has been shown in the preceding section, the presence of procarpic branches and cystocarps is not in itself evidence of the haploid nature of the plant on which they are borne. In order to complete the account of the life-

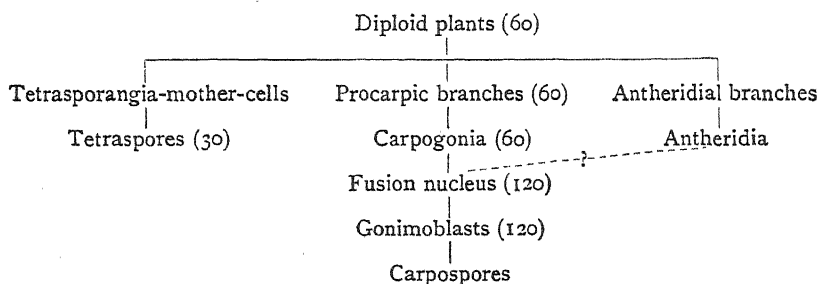
history of this alga, collections at other seasons of the year are much to be desired.

#### DISCUSSION.

The observations recorded in the preceding pages show that sexual organs and cystocarps, as well as tetrasporangia mature on diploid plants of *Spermothamnion Turneri*. As in all other tetraspore-bearing Florideae, which have been investigated cytologically, the first nuclear division in the tetrasporangium is a reduction division, resulting in the formation of haploid tetraspores. The diploid procarpic branches and cystocarps develop in the normal way, but as there is no reduction division during the formation of the procarpic branches the carpogonia are diploid. Moreover since a nuclear fusion between the egg-nucleus and a second nucleus (probably spermatial in origin) initiates the development of the cystocarps, nuclei found in division in the resulting gonimoblasts, are, with one exception, tetraploid. The exception is triploid.

Diploid plants of this species therefore give rise to two kinds of reproductive spores, haploid tetraspores and tetraploid carpospores. No evidence of the viability of the carpospores has been obtained, but the tetraspores germinate readily and provide evidence<sup>1</sup> of the existence of a haploid generation.

These results are collected together in the accompanying diagram and the chromosome number is indicated where it has actually been ascertained.



Until it is known whether tetraploid carpospores germinate (and if so, what reproductive organs are borne on the mature individuals) and also whether tetrasporangia as well as sexual organs develop on the haploid plants, the life-history of *S. Turneri* will remain incompletely known. No reason has been found for supposing that the cytological alternation of haploid and diploid generations is any less strict in this species than in *Polysiphonia violacea*, for example, for although no mature haploid plants have been found, the tetraspores germinate readily. At the same time, until it is shown that the tetraploid carpospores are not viable, the possi-

<sup>1</sup> Further evidence of the existence of a haploid generation is provided by the occurrence of a haploid nucleus in a trichogyne of a procarpic branch and also indirectly by a triploid gonimoblast.

bility of another alternation of tetraploid and diploid generations cannot be ignored. Such an alternation would account for what seems to be a preponderance of diploid plants.

This species is also of interest in that there is no strict segregation of tetrasporangia and functional sexual organs to separate individuals. Thus there is no morphological alternation of sexual and asexual generations corresponding to the haploid and diploid states. On account of this peculiarity, *S. Turneri* might be considered either as an aberrant member of the diplobiontic Florideae or as a form illustrating an evolutionary step in the Florideae as a whole. Accepting the view that the ancestral Florideae were haplo-biontic and that the one individual possessed both sexual organs and accessory spores, *S. Turneri* would then take its place as a primitive diplobiont, in which the reduction division has been postponed from the carpogonium of one generation to the accessory spores of the next. The sporangium undergoes a protoplasmic division in consequence. With the further step of a segregation of sexual organs and sporangia to separate plants, the usual diplobiontic life-cycle would be reached.

The fact that the diploid sexual organs are functional shows that the dominating factor which determines this, is not to be found in the chromosome number. In this way, *S. Turneri* provides a parallel in the algae to *Osmunda regalis* (14) and some members of the Bryophyta (26), where diploid gametophytes give rise to tetraploid sporophytes. The difference between the Archegoniatae and *S. Turneri* lies in the fact that in the former the diploid is a normal gametophyte except for its chromosome number, but in the latter both kinds of reproductive organs occur together.

Reference has been made to an investigation by Schussnig and Odle (20) of *Spermothamnion roseolum* (Ag.) Pringsh., which is either very nearly related to *S. Turneri* or possibly another form of the same species. These authors conclude that although tetrasporangia occur on the sexual plants and sexual organs on the tetrasporic plants, that the alternation of generations (Generationswechsel) is normal. They find that the procarpic branches and spermatangia on the tetrasporic plants never get beyond the first stages of development, and the tetrasporangia on both tetrasporic and sexual plants are morphologically identical. Also, reduction division takes place in the tetrasporangia of the former only. The nuclear divisions in the haploid tetrasporangium are not described, however. As no mature haploid plants of *S. Turneri* have been found, no comparison is possible with regard to that generation or to the life-cycle as a whole. Whereas in *S. roseolum*, the diploid sexual organs do not mature, the preceding account shows that those of *S. Turneri* are capable of doing so and give rise to tetraploid carpospores.

A lengthy discussion between Kylin (11, 12) and Schussnig (21, 22) has resulted from the publication of Schussnig and Odle's work in 1927 (20).

The point of attachment and position of the carpogonial branch, the number of cells in that branch, the position of the auxiliary cells, and their number and time of their formation have been the chief points of structure discussed. In so far as *S. Turneri* has any bearing on this discussion, the present investigation undoubtedly confirms what Kylin has recorded and is in disagreement with the findings of Schussnig and Odle. The carpogonial branch of *S. Turneri*, although attached to the supporting-cell, comes to occupy an adaxial position when mature, due to the shape of the lowest cell of the branch. The branch is invariably four-celled. There are two potential auxiliary cells, but in cases where only one sporogenous cell develops, only one auxiliary cell functions. They are never formed before nuclear division starts in the carpogonium.

A comparison of the cytological details recorded in this investigation with those known for other Florideae, shows that the method of division of the vegetative nuclei of *S. Turneri* agrees closely with most of these. It differs from *Griffithsia Bornetiana* (13) and others in that the nucleolus does not directly and obviously provide any material for the elaboration of the chromosomes. However, like that species and unlike many other Florideae, the spindle is intranuclear. No spindle fibres, kinoplasmic caps, or centrospheres have been seen. Schussnig and Odle (20) do not describe the details of the division of the somatic nuclei of *Spermothamnion roseolum*.

Detailed accounts exist of the nuclear divisions, and particularly the reduction division, in the tetrasporangium of several Florideae (3, 7, 8, 13, 23, 27). These do not agree in detail amongst themselves, so it is not surprising that the preceding account does not conform very closely with most of them. At the same time, the general outline and many of the details of the nuclear divisions in the tetrasporangium of *S. Turneri* parallel those given by Kylin for *Griffithsia corallina* (8) and *Rhodomela virgata* (7). In some of the species investigated, such as *Griffithsia Bornetiana* (13), *Delesseria sanguinea* (23), and *Spermothamnion roseolum* (20), the nucleolus supplies material for the formation of the chromosomes, but in *S. Turneri* the nucleolus remains intact until the chromosomes are completely formed, and at no stage is there any trace of chromatin bodies detaching themselves from the nucleolus. Although no definite centrosomes have been found, polar caps of kinoplasm, very like those recorded for *Delesseria sanguinea* (23) and *Rhodomela virgata* (7), occur during the first division. Whereas in *R. virgata* they are not visible after metaphase, they persist in *S. Turneri* throughout the nuclear division and the daughter nuclei are reorganized in them. As in the vegetative divisions, the spindle of the reduction division is intranuclear, although this is not the case in the majority of the Florideae.

The close resemblance of the nuclear behaviour, subsequent to fer-

tilization, between *S. Turneri* and *Callithamnion corymbosum* (16) is worth noting. Unlike *C. corymbosum*, however, no cell division follows the first nuclear division in the carpogonium of *S. Turneri*, and the second nucleus of each sporogenous cell remains in situ, instead of being cut off in the foot cell with the nucleus of the auxiliary cell. This is probably due to the fact that the sporogenous and foot cells are not adjacent as in *C. corymbosum*, but on diagonally opposite sides of the central cell in *S. Turneri*.

#### SUMMARY.

1. Diploid plants of *Spermothamnion Turneri* (Mert.) Aresch., bearing sexual organs and cystocarps in addition to tetrasporangia, have been investigated cytologically.

2. The division of the somatic nuclei has been followed, the diploid number of the chromosomes being sixty, an unusually high number for the Florideae.

3. A study of the nuclear history of the developing tetrasporangium shows that a reduction division precedes the formation of the tetraspores on diploid plants.

4. Tetraspores germinate very rapidly in the laboratory, but growth stops when the germlings are three- or four-celled. These germlings, the nuclei of which contain the haploid number of chromosomes, provide most of the evidence yet available, of a haploid generation.

5. Procarpic branches formed on the diploid plants develop in the normal manner and no reduction division precedes the formation of the carpogonium, which is therefore diploid.

6. The fusion of the egg-nucleus with a second nucleus (probably spermatial in origin) initiates the development of the cystocarp. This proceeds normally and the gonimoblasts are tetraploid. (A single exception was found to be triploid.)

7. No evidence has been found which would suggest that the cytological alternation of haploid and diploid generations is any less strict in this species than in *Polysiphonia violacea*, for example, but until it is shown that the tetraploid carpospores are not viable, the possibility of an alternation of tetraploid and diploid generations cannot be ignored. Since procarpic branches mature on the diploid plants and subsequently give rise to cystocarps, there is no strict morphological alternation of sexual and asexual generations, corresponding to the haploid and diploid states, in this species.

8. The division of the somatic nuclei is essentially similar to that described for other Florideae. The reduction division in the tetrasporangium, although very like that in *Griffithsia corallina* (8) and *Rhodomela virgata* (7), differs somewhat from the same process in other species of the

red algae. Although minor differences exist, the nuclear behaviour in the developing cystocarp agrees with that of *Callithamnion corymbosum* (16).

In conclusion, the writer wishes to express her thanks to Professor W. H. Lang for his constant help and interest throughout this investigation, and to Dr. W. R. Taylor for advice during the collection of the material. Thanks are also due to the Council of the University of Manchester for laboratory facilities, and to the Commonwealth Fund of New York for providing the opportunity of visiting the United States, where the material was collected.

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## EXPLANATION OF PLATES XII AND XIII.

Illustrating Mrs. Baker's paper on 'Contributions to the Cytology of *Spermothamnion Turneri*. (Mert.) Aresch. I. The Diploid Generation.'

### PLATE XII.

- Fig. 1. Resting vegetative nucleus.  $\times 2,350$ .
- Fig. 2. Late prophase of somatic nuclear division in slender apical cell. Sixty chromosomes present around nucleolus, but four not visible in the figure.  $\times 2,350$ .
- Fig. 3. Later stage, showing chromosomes moving to equatorial plate.  $\times 2,350$ .
- Fig. 4. Polar view of metaphase in somatic nucleus.  $\times 2,350$ .
- Fig. 5. Side view of same.  $\times 2,350$ .
- Fig. 6. Anaphase showing fibres connecting daughter chromosomes.  $\times 2,350$ .
- Fig. 7. Telophase.  $\times 2,350$ .
- Fig. 8. Reorganization of one of daughter nuclei.  $\times 2,350$ .
- Fig. 9. Vegetative cell with nuclei in division. Basal nuclei at metaphase, middle ones at anaphase, and two superimposed apical ones at telophase.  $\times 1,200$ .
- Fig. 10. Cell about to segment into tetrasporangium-mother-cell and stalk-cell.  $\times 1,200$ .
- Fig. 11. Nucleus of very young tetrasporangium-mother-cell.  $\times 2,350$ .
- Fig. 12. Nucleus of slightly older tetrasporangium-mother-cell in synizesis.  $\times 2,350$ .
- Fig. 12 a. Loop of thread from same nucleus.  $\times 2,350$ .
- Fig. 13. Nucleus showing segmentation of spireme to give bivalent chromosomes.  $\times 2,350$ .
- Fig. 13 a. Portion of spireme from same nucleus.  $\times 2,350$ .
- Fig. 14. Nucleus with bivalent chromosomes distinctly separate and their double nature becoming apparent.  $\times 2,350$ .
- Fig. 15. Nucleus enlarging and chromosomes shortening, but members of each chromosome pair more separate.  $\times 2,350$ .
- Fig. 16. Slightly later stage, showing further contraction of the chromosomes, which number thirty. One is under the nucleolus.  $\times 2,350$ .
- Fig. 17. Nucleus showing the bivalent chromosomes of diakinesis. Fission of some of the univalent chromosomes is beginning, as at  $\alpha$ . Chromosomes number thirty, two of which are under the nucleolus. Structure marked  $\alpha$ , probably extra-nuclear.  $\times 2,350$ .
- Fig. 18. Nucleolus disintegrating. Thirty chromosomes so contracted that the bivalent nature of the majority is no longer visible.  $\times 2,350$ .

Fig. 19. Early metaphase of first meiotic division. Some chromosomes already showing signs of disjunction. Nucleus smaller but membrane still present.  $\times 2,350$ .

Fig. 20. Later metaphase with the chromosomes in the process of disjunction. One pair ( $\alpha$ ) has separated completely. Polar caps of kinoplasm ( $\phi$ ) visible and nucleus still smaller.  $\times 2,350$ .

Fig. 21. Anaphase. Two groups of chromosomes moving apart, but two members of some of the pairs still connected by a fibre. Two chromosomes in disjunction on plate. Twenty-five chromosomes distinguishable in upper and twenty-seven in lower group. Nuclear membrane practically gone at equator. Polar caps enlarging and centres of same becoming clear.  $\times 2,350$ .

Fig. 22. Telophase. Chromosomes have fused together and entered the polar caps.  $\times 2,350$

Fig. 23. Reorganization of daughter nuclei.  $\times 2,350$ .

Fig. 24. Daughter nuclei in interphase.  $\times 2,350$ .

Fig. 25. Prophase of homotypic division in sporangium. Nuclei have enlarged but are smaller than the nucleus of the corresponding stage of the first division. Chromosomes, which are also smaller and showing signs of fission, number thirty in both nuclei, but one in upper nucleus not visible in the figure.  $\times 2,350$ .

Fig. 26. Metaphase of second nuclear division, showing one plate in surface and the other in side view. Plates small and nuclear areas not well defined.  $\times 2,350$ .

Fig. 27. Telophase of one nucleus of homotypic division. Chromosomes fused together into mass.  $\times 2,350$ .

Fig. 28. Nucleus of tetrasporangium-mother-cell nearing diakinesis. Two bodies marked  $\alpha$ , probably extranuclear, leaving thirty bivalent chromosomes.  $\times 2,350$ .

Fig. 29. Prophase of nucleus of stalk-cell of sporangium borne on same filament as sporangium of Fig. 21. Chromosomes number fifty-five, but one is hidden completely by another.  $\times 2,350$ .

Fig. 30. Sporangium with multinucleate spores.  $\times 410$ .

#### PLATE XIII.

Fig. 31. Two-celled procarpic branch; nucleus of apical cell in prophase.  $\times 1,200$ .

Fig. 31 a. Nucleus of apical cell of same. Fifty-nine chromosomes present, but three are beneath the nucleolus.  $\times 2,350$ .

Fig. 32. Young procarpic branch with two pericentral cells; nucleus of second ( $\phi c 2$ ) in prophase.  $\times 1,200$ .

Fig. 32 a. Nucleus of second pericentral cell of same. Chromosomes number sixty, three of which are hidden beneath others.  $\times 2,350$ .

Fig. 33. Slightly older procarpic branch with sterile cell ( $st c$ ) cut off from supporting cell ( $sc$ ). Nucleus of central cell ( $cc$ ) in prophase prior to formation of third pericentral cell.  $\times 1,200$ .

Fig. 33 a. Nucleus of central cell of same. Four of sixty chromosomes present hidden behind the nucleolus.  $\times 2,350$ .

Fig. 34. Procarpic branch with immature three-celled carpogonial branch; nucleus of apical cell of branch in prophase.  $\times 1,200$ .

Fig. 34 a. Nucleus of apical cell of carpogonial branch. Sixty chromosomes present, but three are completely covered by others.  $\times 2,350$ .

Fig. 35. Procarpic branch with four-celled carpogonial branch. Nucleus of third cell of branch ( $cp br 3$ ) in prophase.  $\times 1,200$ .

Fig. 35 a. Nucleus of third cell of carpogonial branch. Chromosomes number fifty-seven, but one is beneath the nucleolus.  $\times 2,350$ .

Fig. 36. Similar procarpic branch viewed from the opposite side. Nucleus of carpogonium ( $cp$ ) in prophase prior to formation of trichogyne nucleus.  $\times 1,200$ .

Fig. 36 a. Nucleus of carpogonium. Chromosomes number fifty-nine, two of which are completely hidden beneath others.  $\times 2,350$ .

Fig. 37. Apical cell of filament of Text-figure 1 c with nuclei in prophase. Procarpic branch of Fig. 33 borne on same filament.  $\times 1,200$ .

Fig. 37 a. Central nucleus of apical cell. Fifty-eight chromosomes distinguishable, but two are completely covered by others.  $\times 2,350$ .

Fig. 38. Nucleus of sporangium of filament of Text-figure 1 a with thirty bivalent chromosomes, one of which is beneath the nucleolus. Procarpic branch of Fig. 36 borne on same filament.  $\times 2,350$ .

Fig. 39. Procarpic branch after nuclear fusion in carpogonium (*cp*). Carpogonial branch is on the back side of the procarp, beneath the auxiliary cell (*ac* 2) and its basal cell (not indicated), and the central cell of the procarp (indicated by broken line). On the right of the procarp and in front is the first pericentral cell (*pc* 1) and behind it is the auxiliary cell (*ac* 1) and its basal cell (*bc*), formed by division of the supporting cell. Behind the auxiliary cell is the sterile cell (*sc*) (indicated by a broken line). The two nuclei of the carpogonium, the two in each of the other three cells of the carpogonial branch, the nucleus of the one auxiliary cell (*ac* 1), and that of its basal cell are the only ones represented.  $\times 1,200$ .

Fig. 39 a. Nucleus of carpogonium in prophase. Of one hundred and eleven chromosomes distinguishable, eleven are completely hidden by others.  $\times 2,350$ .

Fig. 39 b. Nucleus of basal cell (derived from supporting cell) of same procarpic branch in prophase. Chromosomes distinguishable number fifty-six or fifty-eight, two of which are not visible in this figure.  $\times 2,350$ .

Fig. 40. Procarpic branch showing further changes following nuclear fusion in the carpogonium. Cells on back side not indicated. Sporogenous cells (*sp* c) formed and in communication with central cells (*gc*), from which foot cells (*fc*) are about to be cut off. Two degenerating nuclei in carpogonium, large multinucleolate nucleus in each central cell, and similar one in each sporogenous cell.  $\times 1,200$ .

Fig. 41. Triploid nucleus from cell of gonimoblast. Eighty-two or eighty-three chromosomes distinguishable, but twelve of these are covered by others.  $\times 2,350$ .

Fig. 42 a. Nucleus from apical cell (*x*) of involucreal filament of developing cystocarp of Text-figure 2 a. Chromosomes number fifty-nine or sixty, but three are completely hidden by others.  $\times 2,350$ .

Fig. 42 b. Nucleus in prophase, from cell of gonimoblast of same developing cystocarp. Of one hundred and ten chromosomes present, ten are not visible in the figure, being covered completely by others.  $\times 2,350$ .

Fig. 43. Carpogonial branch showing haploid nucleus (*n*) in trichogyne. Two other degenerating nuclei present.  $\times 1,200$ .

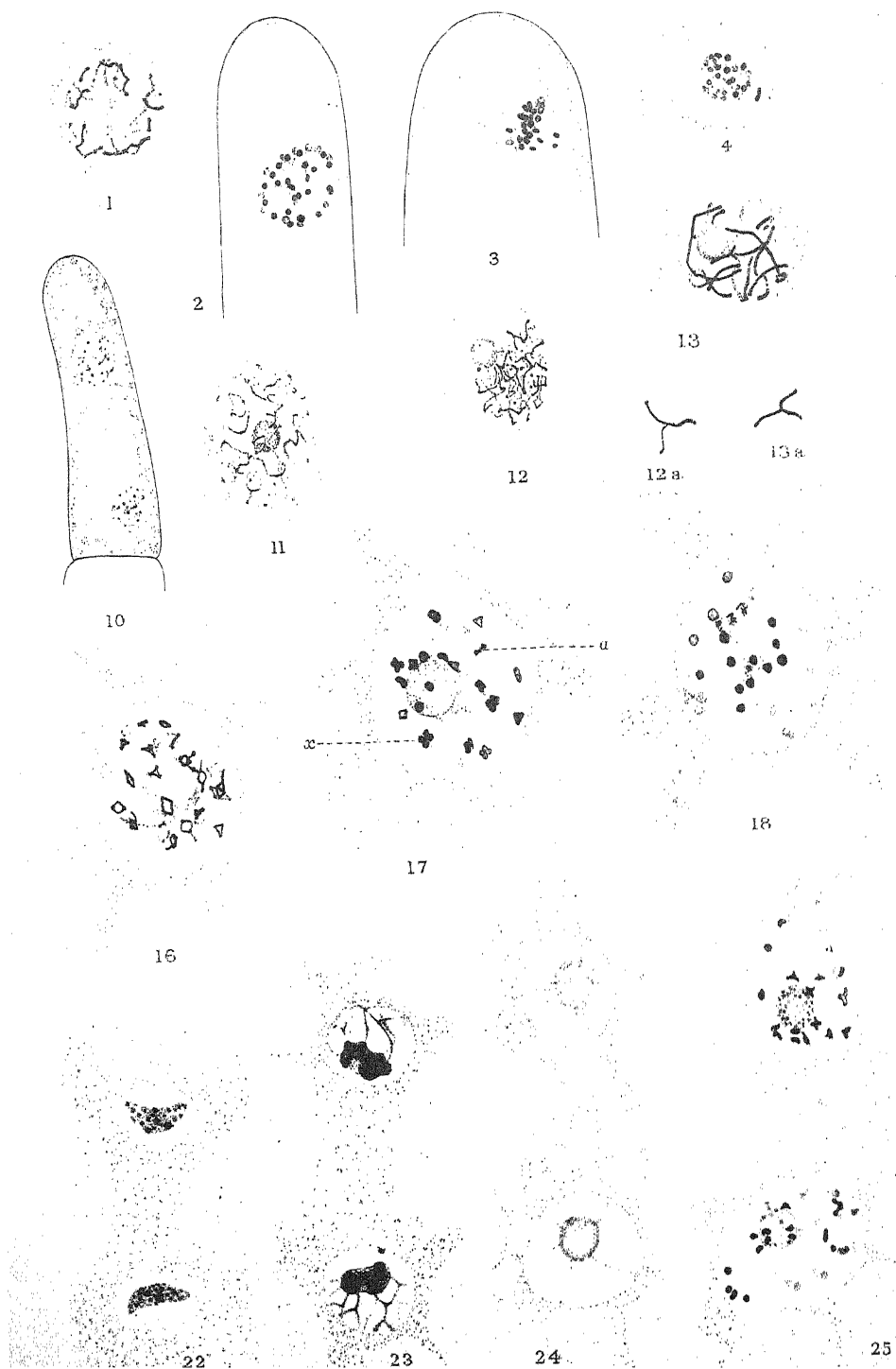
Fig. 43 a. Haploid trichogyne nucleus in detail. Thirty-one chromosomes present.  $\times 2,350$ .

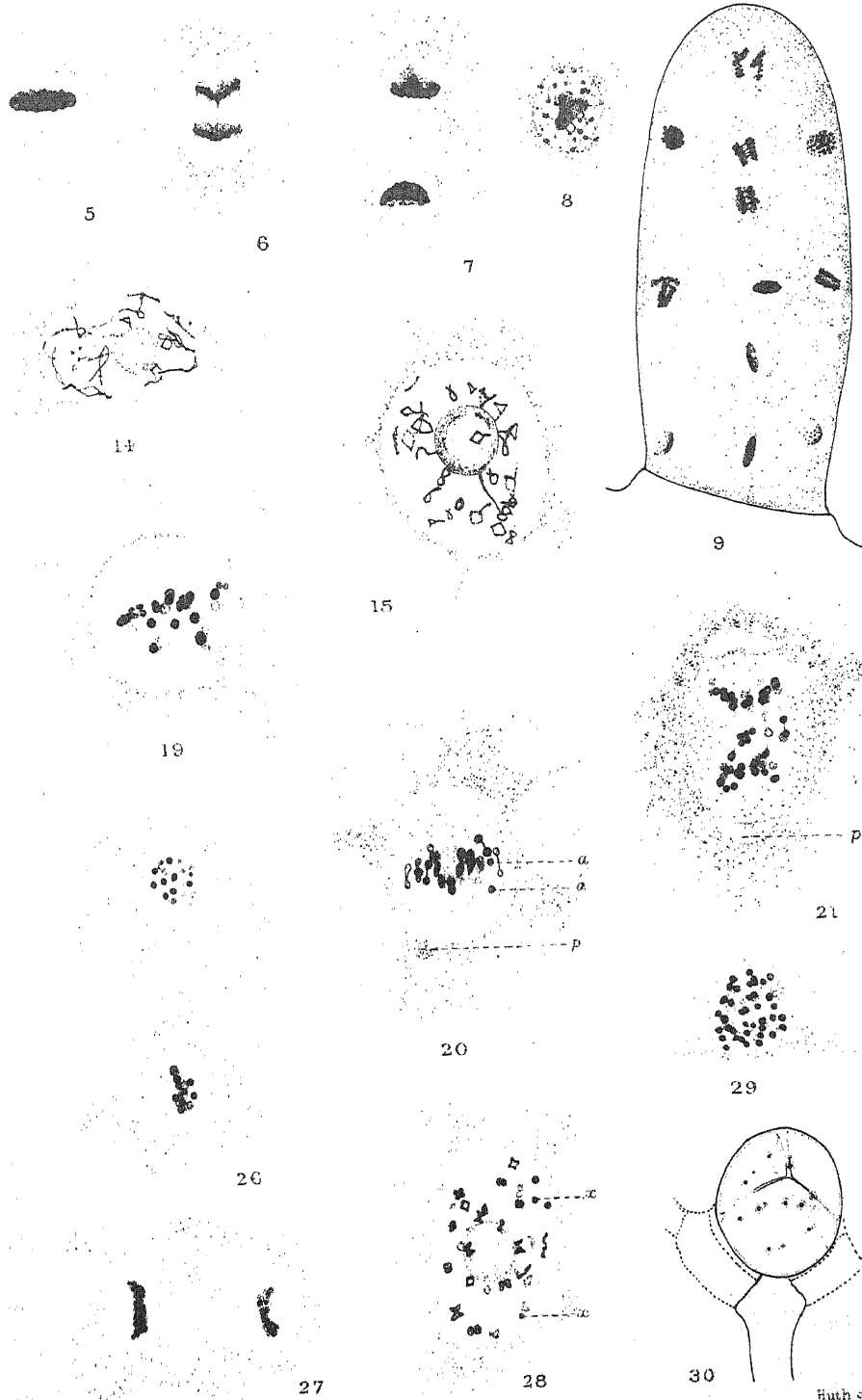
Fig. 44. Procarpic branch with multinucleate trichogyne.  $\times 900$ .

Fig. 45. Procarpic branch with trichogyne dividing into several cells.  $\times 900$ .

Fig. 46. Germinating tetraspore with nuclei of upper cell in prophase.  $\times 900$ .

Fig. 46 a. One of these nuclei in detail. Of thirty chromosomes present, one is not visible in the figure.  $\times 2,350$ .



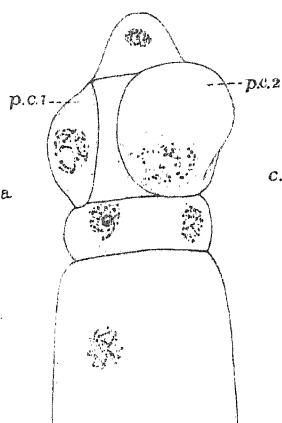




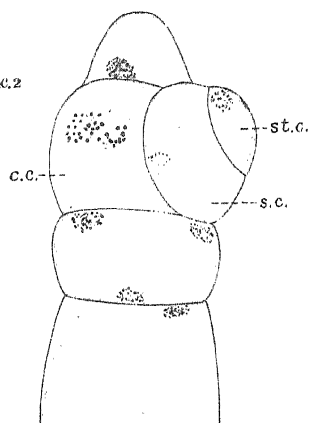
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31a



32



33



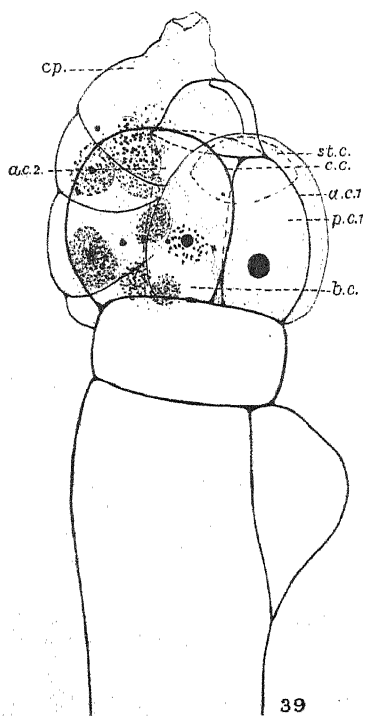
39a



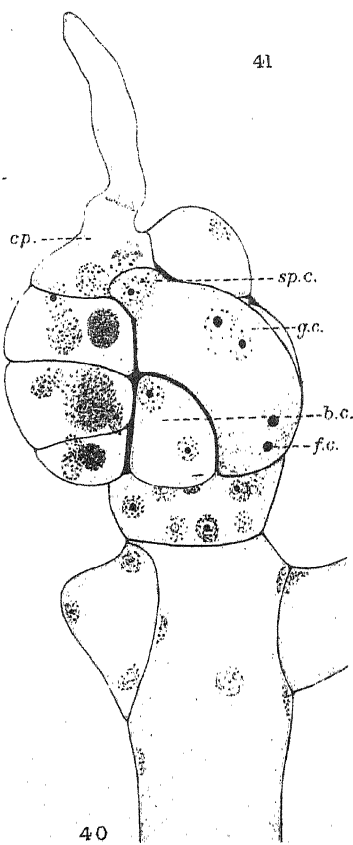
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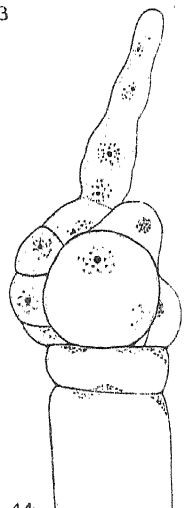
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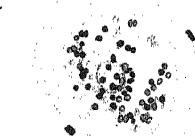
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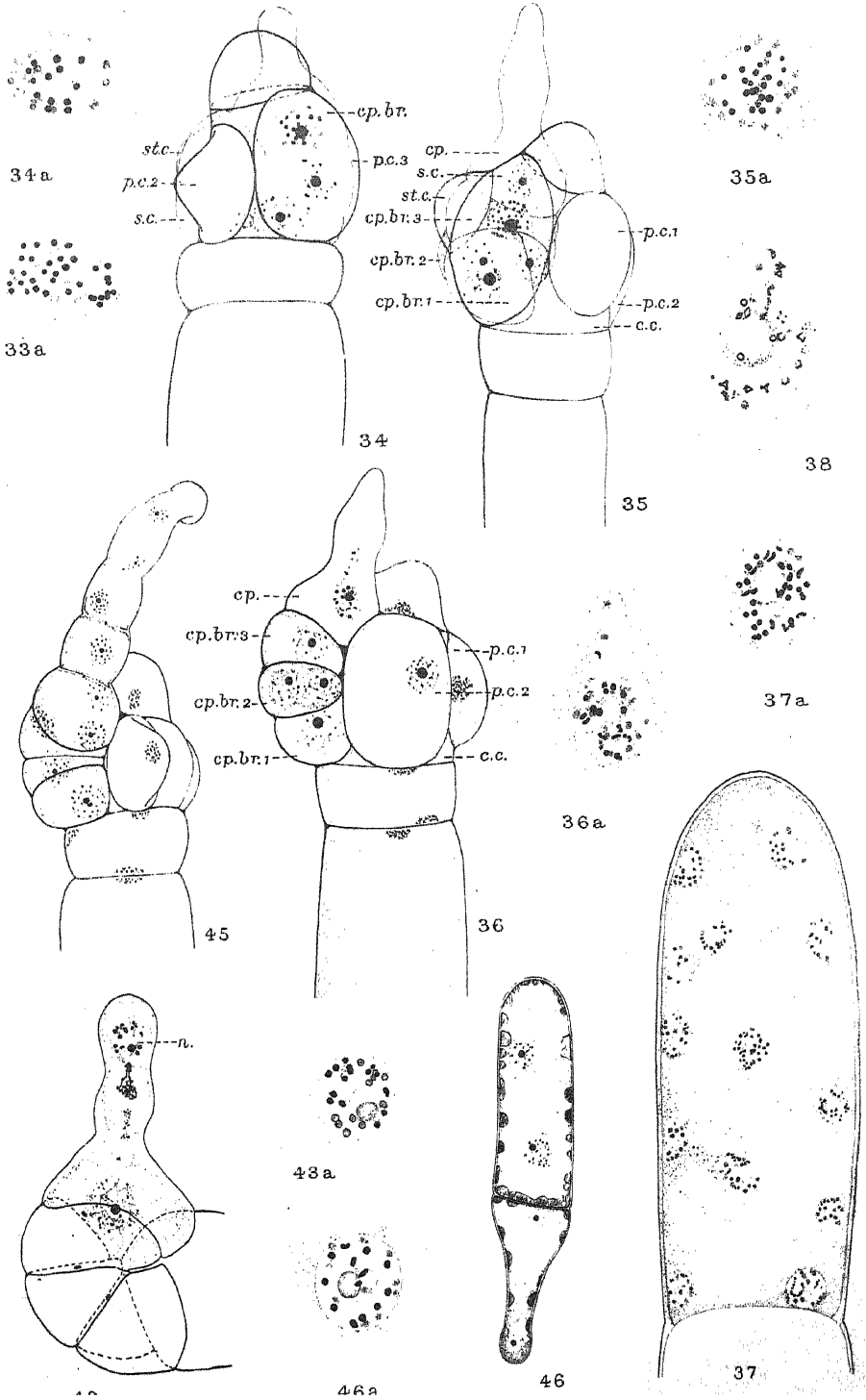
44



42a



42b







# On the Effects of Partial Removal of the Cotyledons upon the Growth and Duration of Life of Canteloup Seedlings without Exogenous Food.<sup>1</sup>

BY

SOPHIA A. GOULD, RAYMOND PEARL, THOMAS I. EDWARDS

AND

JOHN R. MINER.

With six Figures in the Text.

## I. THE PROBLEM.

IN a series of studies carried out in this laboratory during a period of more than ten years, there have been used as experimental material seedlings of the canteloup (*Cucumis melo*), grown under rigorously controlled environmental conditions. These conditions were so arranged that the growing plant could obtain energy and matter (other than water and air) for the purposes of metabolism, *only* from endogenous sources. The experimental methods have been described in some detail in (17) and (18). Briefly, they consist of an arrangement by which the plant grows and lives out the remainder of its life after growth has been completed, in a long glass tube, in sterile (aseptic) surroundings. The roots of the seedling penetrate, and are surrounded by, a solid (gel) sterile medium, composed solely of purified agar, from which all water-soluble material has been removed by repeated washing in distilled water. The only other constituent of the medium is distilled water. The seedlings are grown in the dark, and observed and measured only in non-actinic light, as in a photographic dark room, so that photosynthesis cannot take place. Thus all the energy and material (other than water and oxygen) metabolized by the plant come from the store of reserve food laid down in the cotyledons of the seedling itself. The gross morphological features of growth, and the duration of life of seedlings grown under the conditions just described, have been studied

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in great detail, and as many of the results as it has been possible to prepare for publication up to this time, have been published (16-23).

It is desirable at this point to review briefly the important gross morphological features of the life-history of seedlings of *C. melo* in the absence of exogenous nourishment. The seed from which the testa has been removed is sterilized by immersion in 1:1,000 bichloride of mercury solution, and placed, with aseptic precautions, on the surface of the purified, sterile agar in the growing tube. Germination takes place during the next twenty-four hours. In the seedling with normal response to gravitation (a few appear to lack this in early stages or to have it only in weak form) the root-tip soon pushes its way down into the agar, and the hypocotyl bearing the cotyledon at its upper end begins growing up the tube. Under the described experimental conditions the epicotyl never develops. From the point of view of analytical experimentation this is a great advantage, because there are only two organ systems, hypocotyl and roots, competing for nourishment. The hypocotyl grows in length according to a logistic curve (22, 23). A richly branched root system develops in the agar. The seedling ceases growing after about twelve to fifteen days (at 25° C.). The portion of the seedling's life up to this point may conveniently be designated as the *period of growth* (Fig. 1, A, B; Fig. 2, A). This is the time of the seedling's youth.

Following the termination of the growth period the seedling passes into the second stage of its life cycle, which may conveniently be called the *intermediate period*<sup>1</sup> (Fig. 1, C, and Fig. 2, B). During this period the plant is alive and carrying the function of respiration and metabolism generally. We have many observations (as yet unpublished) on the rate of metabolism in this period. The cells maintain themselves in full turgor. The plant presents the appearance of perfect health and vigour. The duration of this intermediate period varies among different individual seedlings, but is roughly, on an average, of about the same order as the duration of the growth period. This is the part of the seedling's life cycle, under the described experimental conditions, which roughly corresponds to adult life in the whole life cycle under natural conditions.

The termination of the intermediate period is marked by visible external evidences of the beginning of a series of physical and chemical changes in the plant which continue without interruption until the whole is dead. This part of the life cycle may be conveniently designated as the *period of disintegration*<sup>2</sup> (Fig. 1, D and E, and Fig. 2, C). Disintegration

<sup>1</sup> This terminology implies merely that we are dealing with a part of the seedling's life cycle which falls, *in time*, between the period of growth on the one hand and the period of disintegration on the other hand.

<sup>2</sup> Using the word in its literal sense. The normal biological integration of the cells, and of the plant body as a whole, is visibly altered as the processes leading to death begin.

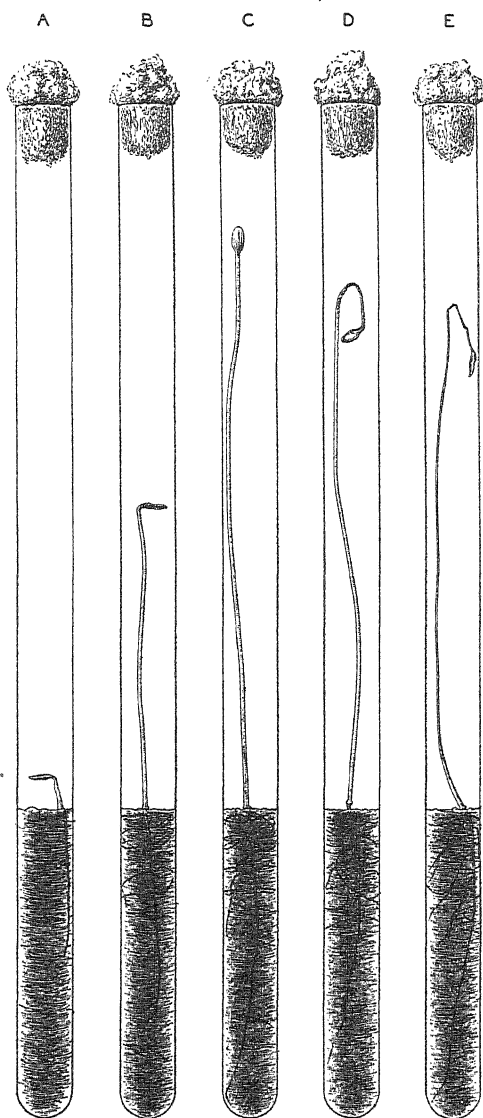


FIG. 1. Seedlings of *Cucumis melo* grown in the absence of exogenous nourishment. Each drawing shows a tube containing the purified agar medium at the bottom, in which the roots are growing, and above which is the straight upright hypocotyl, bearing the cotyledon at its upper end. A. Seedling in early stage of growth period. B. Seedling in late stage of growth period. C. Seedling in intermediate period. D. Seedling in early stage of period of disintegration (beginning of death). E. Seedling in late stage of period of disintegration. (All figures reduced.)

usually begins, under the conditions of the experiments, at the top of the hypocotyl, just below the cotyledon, and continues progressively downwards. In some plants, however, the external signs of disintegration may begin at the lower end of the hypocotyl. Usually the first evidence that the seedling

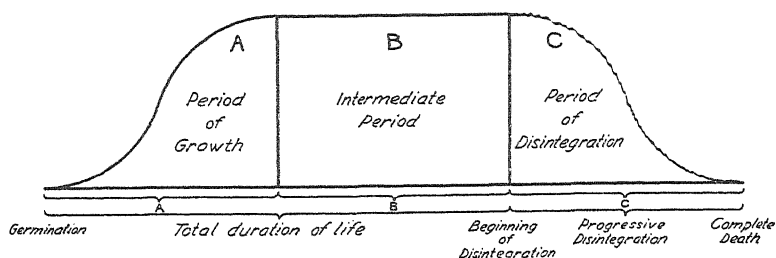


FIG. 2. Diagram of the life cycle of a seedling of *Cucumis melo* under conditions described in the text (absence of exogenous nourishment). A. Growth period. B. Intermediate period. C. Period of disintegration and death.

is beginning to die is the appearance of fluid in fairly large drops along the hypocotyl. Following this the stem becomes translucent, losing the ivory white opacity characteristic of healthy seedlings. It finally becomes limp and watery along its entire length and ultimately collapses to the bottom of the tube. The whole appearance is that of a gradual, but continuous, sterile autolysis of the hypocotyl. In some cases the first symptom of beginning disintegration is the shrinking of some portion of the hypocotyl or cotyledons in diameter, but without the appearance of fluid on the surface. The shrunken area gradually extends along the hypocotyl and finally, just a day or two before the complete collapse, fluid does appear just as in the previous type of morbid change, and the end is general autolysis. The only end point which is definite enough to be useful in defining the end of this intermediate period is the appearance of the first symptoms of death.

The whole life cycle of a seedling of *C. melo*, so far as concerns its external morphology, then may be diagrammatically represented as in Fig. 2. The embryo first grows (A) for a certain time and to a certain amount, both of which appear to be self-limited (i.e. by the internal organization of the plant, operating under the described conditions); it then lives for a time (B) in a steady state of equilibrium with its environment; it then begins to disintegrate and is finally completely dead (C). During the periods A and B it is physiologically doing three things of major importance, viz. (1) drawing food materials from the cotyledons, (2) drawing water from the substrate through the roots, and (3) respiring. What is going on physiologically in period C is imperfectly understood, and in fact constitutes one of the major problems of our investigations.

Under the experimental conditions just described the growth of the seedling and its duration of life should theoretically be proportional to the amount of available food stored in the cotyledons. The primary problem of the present paper is to determine how strictly this relationship holds true. Specifically the questions we are asking are :

1. If a portion of the cotyledons is removed surgically, will the performance of the plant in respect of growth and duration of life, be in all ways strictly proportional to the amount of the cotyledons left after the operation, the conditions of the experiments being such that no exogenous nourishment is available ?

2. If not, what is the manner and degree of deviation from such strict proportionality ?

Various workers have studied the dependence of the plant embryo upon endogenous and exogenous food materials in a quantitative way. The oldest method of study, that of Malpighi (12) and of Bonnet (2), was to remove the cotyledons of seedlings early in the germination process. Bonnet, for instance, noted that buckwheat plants thus deprived of their cotyledons were one-sixth the height of untreated plants after three weeks of growth and the same relationship persisted until the time of flowering.

Later workers, notably Sachs (25), van Tieghem (27), Haberlandt (8), Meanard (15), Delassus (5, 6), and Dubard and Urbain (7), reduced the available food supply of the embryo by cutting away only a part of the endosperm or cotyledons, and grew such treated plants in comparison with untreated controls. Such experiments were carried out with small numbers of seeds ; usually with four degrees of mutilation at most ; growth records were meagre ; and the plants were grown in light. Sometimes the conditions were such that removal of part of the cotyledons not only reduced the food reserves but also reduced the assimilating surface, which complicates the problem by reducing the food supply in two ways. An excellent résumé of the literature in this field up to 1919 was prepared by Kidd and West (11) and on that account it is not necessary to discuss the earlier work in detail here. They say of this work : ' The main facts which emerged from their results were, that the life-duration of the embryo plant in the absence of other food supplies was directly related to the amount of food material supplied from the cotyledon or endosperm, and that the size of all the organs developed also bore a direct relation to the amount of food material originally available.'

The work of Hamada (9) marks an advance in the analysis of the problem. He (*loc. cit.*, p. 179) removed half of the endosperm from germinating oat seeds and allowed them to develop in darkness at 25° C. together with controls. At twenty-four-hour intervals for eight days, samples of thirty to forty seedlings were removed and the lengths of mesocotyl, coleoptile, and primary leaf were measured. For the first two organs the

relation of length to age was represented by sigmoid curves which approach an upper growth limit asymptotically. The seedlings from whole grains elongated faster during the latter part of the growth cycle than did those from mutilated seeds and attained a greater final length. The data do not show any significant differences in length of time required for cessation of growth. During the period of time covered by his observations the growth rate of the primary leaf increased steadily, the mutilated seedling having the slower rate. Of the three organs, the mesocotyl, the first to develop, was least affected by the decrease in amount of reserves and the primary leaf was most affected.

In one experimental series Shear (26) deprived peanut embryos of their cotyledons before germination, culturing the embryos on agar in darkness at 23°. In another series the cotyledons were not removed until the sixth day of growth, the plants being grown in sand at the same temperature as before. He says of these (p. 282): 'The embryos with excised cotyledons grown to find out how long they were capable of living, probably died from desiccation rather than inanition. One, however, lived for one hundred and forty days before the agar dried up. This, when compared with the length of life (some twenty days) of peanut seedlings whose cotyledons were removed after six days' growth, shows that the rate of metabolism at the time that starvation is induced has a marked effect on the length of life. The embryos are able to utilize their small reserve of food much more economically than seedlings with a much greater reserve.' The increase in height and dry weight of peanut and bean seedlings (exclusive of cotyledons) grown in darkness follows sigmoid curves. Removal of cotyledons early in the growth cycle results in a lessened growth rate and a smaller final height. However, the rate of disappearance of oil is decreased also to such an extent as to make it appear that the food reserves are being consumed more efficiently than in the untreated control seedlings. A second type of study is represented by the culture of isolated seed embryos on nutrient media of varying concentration after the manner of Andronescu (1), Brown and Morris (3), and Buckner and Kastle (4). So far, the growth measurements in studies of this kind have been so limited that they do not have much bearing on the problem under consideration here.

## II. METHODS.

The cultural methods used in this experiment are identical with those used in other previously reported experiments carried out in this laboratory with the same material (17), and described in the preceding section. As a consequence the results of the several studies are directly comparable with each other.

In the present series of experiments, 145 *C. melo* seeds of approximately the same weight were selected from the same melon to insure as uniform a genetic constitution as possible. After dry storage the testas were removed under aseptic conditions by gentle pressure applied with forceps. The shelled seeds were then sterilized by immersion for one minute, with stirring, in a 1:1,000 solution of mercuric chloride, followed by rinsing in sterile distilled water, in such a way that each seed was not in contact with any other seed. Previous work (21) has shown that this procedure, followed by incubation on agar, is an especially advantageous way of obtaining a uniform lot of seedlings for experimentation. Ten groups of ten seeds each were taken at random, and in each group as nearly as possible the same proportion of the cotyledons was cut away with a sterile safety razor-blade, under aseptic precautions, and discarded, care being exercised to avoid injury to the embryo. In each of the ten groups a different proportion of the cotyledons was cut away, so that a series of operated seeds was available, ranging from the uninjured control seeds to seeds consisting of little more than an embryo and the bases of the cotyledons. Each seed was then individually weighed and transferred, radicle downward, to an agar surface in a culture tube, for incubation in darkness at 30° C. These tubes were 60 cm. in length and 20 mm. in internal diameter, and each contained 40 c.c. of 1 per cent. agar gel made up in distilled water and sterilized in an autoclave. The tubes were loosely stoppered. There is no evidence that the plants suffer from an accumulation of carbon dioxide or an oxygen deficit. Special *ad hoc* experiments have demonstrated that when the tubes are aerated daily, by blowing air under pressure through them, the form and final heights and weights of the seedlings are the same as when the procedure here described is used. It was found in preliminary experimentation that the nucellar membrane tended to adhere to the cotyledons tightly enough to hinder the growth of the plant. For that reason it was removed with a hooked rod after the germination of the seed.

At twenty-four-hour intervals the tubes were examined in a dark room, illuminated only by a photographically inactive ruby light. The height of the hypocotyl was measured in centimetres and tenths by applying a scale to the side of the glass tube. At the conclusion of the experiment each seedling was cut up into cotyledons, hypocotyl, and roots, and after drying to constant weight at 98° C., the weights of each organ were recorded.

While the plan of the experiment was to have ten seedlings in each series, this intention was not completely attained, because of accidents which one normally expects in such experimentation. The failures of the numbers in certain of the series were due to such things as imperfect germination, failure of precise geotropic response, resulting in some of the roots

TABLE I.  
*Summary of Growth Records of Mutilated Canteloup Seedlings.*

Series.	Number of seedlings observed.	Seeds planted.		Mean final hypocotyl length (cm.).	Mean growth period (hours).	Mean intermediate period (hours).	Mean total duration of life (hours).	Mean dry weights (mg.) at end of experiments.			Total.
		Weight range (mg.).	Weight (mg.).					Roots.	Hypocotyl.	Cotyledons.	
1	9	1.7-2.4	2.03	1.69	270.7	333.3	604.0	0.22	0.77	0.36	1.35
2	9	2.5-3.4	2.96	3.96	268.0	250.7	518.7	0.30	1.29	0.67	2.26
3	9	3.5-4.4	4.00	4.86	305.3	288.0	593.3	0.42	1.68	0.82	2.92
4	9	4.5-5.9	5.07	6.35	276.0	258.7	534.7	0.50	2.29	0.80	3.59
5	8	6.0-7.4	6.38	7.83	306.0	294.0	600.0	0.67	2.61	0.96	4.24
6	10	7.5-8.9	8.21	8.95	333.6	259.2	592.8	1.08	3.63	1.65	6.36
7	10	9.0-10.9	9.98	9.74	364.8	288.0	652.8	0.78	3.70	1.84	6.32
8	7	11.0-13.9	12.03	10.70	354.9	315.4	670.3	1.14	4.46	2.43	8.03
9	9	14.0-16.9	15.57	12.39	390.2	341.8	732.0	1.60	6.01	2.61	10.22
10	10	17.0-20.2	18.42	13.88	364.8	321.6	686.4	1.71	6.76	3.50	11.97
Controls	4	19.4-21.4	20.37	14.88	360.0	456.0	816.0	1.37	6.72	3.90	11.99



growing in the air and failing to penetrate the agar, failure of the hypocotyl to straighten, &c. Such abnormal seedlings were discarded, so as to leave only normal, typical specimens for the records.

### III. RESULTS.

#### A. *General Observations.*

According to the data presented here, the behaviour of the seedlings was modified greatly by the amount of food materials remaining after the operation. There is no evidence of any sort of shock effect as a result of the operation.

Table I gives raw data observed in the experiments in summarized form. In this table the column headings are self-explanatory, except that it should be understood that by 'mean total duration of life' is meant the average number of hours from the planting of the seed up to the *beginning* of death, i.e. to the time of appearance of the first symptoms of beginning disintegration of the plant. The duration of the intermediate period is from the time the growth of the hypocotyl in length ceases (i.e. the attainment of constant length) to the beginning of death as above defined.

The form of the mean growth curves in each series is shown in Fig. 3.

It is apparent from Fig. 3 that the growth curves in these experiments exhibit the characteristic sigmoid form of the logistic. As a matter of fact logistic curves have been fitted to these observations, and their constants will be discussed in a later section of the paper.

While it is evident that the mean length of the hypocotyl is greatest in the control series; least in Series I, with the greatest amount of cotyledonary material removed; and intermediate between those two extremes in the other series in inverse order to the amount of the cotyledons removed, it is impossible to judge visually from the diagram as to the *proportionality* between total growth of hypocotyl and available food. We may therefore turn to the consideration of:

#### B. *Relative Performance of the Seedlings in Relation to Available Food.*

We shall proceed at once to a direct examination of the answers that the data appear to give to our questions set forth above. In later sections other results of general interest will be discussed.

Table II, which is derived from the raw data of Table I, puts the material in convenient form for the formation of judgements as to whether

the performance of the plants in respect of growth and life duration is proportional to the amount of food material available, and the nature of the deviations from strict proportionality. The plan of Table II is to take

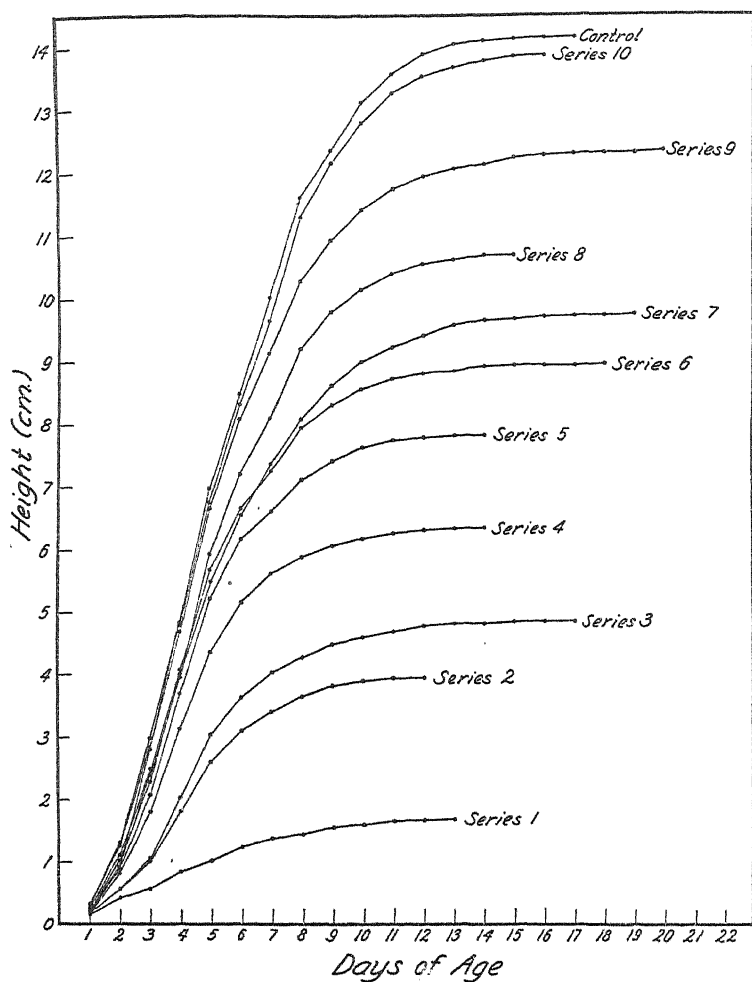


FIG. 3. Growth curves for the several experimental series, showing the mean length of the hypocotyl at each age. The observations are given as circles. Each curve is carried, in the diagrams, only to the point where growth has ceased, i.e. where there is no further elongation of the hypocotyl.

the observed value of the controls as 100 per cent., for each variable, and then express the mean values for each experimental series derived from operated seeds as percentages of the control values. At the beginning of the table stands a column giving relative (percentage) amount (in heavy type) of the seed left after operation in each series (mean values). These

TABLE II.

*Relative Performance of Normal (Control) Seeds, and Seeds from which Different Proportions of the Cotyledons have been removed by Operation. All Figures are Percentages of the Values of the Controls.*

[illegible]

figures indicate the relative amount of food available for the seedling for growth and the continuance of life. Each subsequent column of the table is to be compared with this first one.

From the data of Table II the following points emerge:

1. Excepting the dry weight of the cotyledons at the end of the experiments, no measured character of the plant upon which observations were taken exhibits relative values in the several series even remotely accordant with the proportionate amount of available nourishment in the seeds planted, as shown in the second column of Table II. This statement is true both for growth characters and for duration of life.

2. In all cases, again excepting the final dry weights of the cotyledons, the relative performance of the plant is consistently *greater* than would be expected on the basis of strict proportionality to the amount of food left in the operated seeds planted. Thus, for example, in Series 6, the mean weight of the operated seeds planted was 40.3 per cent. of that of the normal, unoperated, control seeds. But the hypocotyl at the end of the growth period in Series 6 was 63.1 per cent. of that attained by the normal controls; the dry weight of the hypocotyl at the end of the experiment was 54.0 per cent.; the dry weight of the roots was 78.8 per cent.; the dry weight of the hypocotyl + roots was 58.2 per cent., and the dry weight of the whole plant was 53.0 per cent. Furthermore, the mean duration of the growth period in Series 6 was 92.7 per cent. of that in the controls, although the mean weight of the seeds planted was but 40.3 per cent.; the duration of the intermediate period in Series 6 was 56.8 per cent. of that in the controls; and the total duration of life (to the beginning of death) was 72.6 per cent. of that in the controls.

The general results described in 1 and 2 above are of such a striking character that it will be well to examine them in some detail. The significant trends of the results are shown graphically in Fig. 4, where six diagrams are plotted from the data of Table II. These diagrams are all drawn to the same scale and on the same plan. In each case the line along the lower edge of the black area is the graph of the figures in Column (a) of Table II, and represents the mean relative weights of the seeds planted in the several series in the experiments. The line forming the upper edge of the black area in each case is the graph of the column in Table II dealing with the variable indicated in the diagram. The black area, as a whole, then depicts in each case the *excess* in mean relative performance of the plant over expectation, on the basis of relative amount of available food, in respect of the indicated growth or duration of life variable. The several series are spaced along the abscissal axis in accordance with the mean absolute weights of the seeds planted. Where a series showed a relative deficiency below expected performances, the included area is lined instead of being solid black.

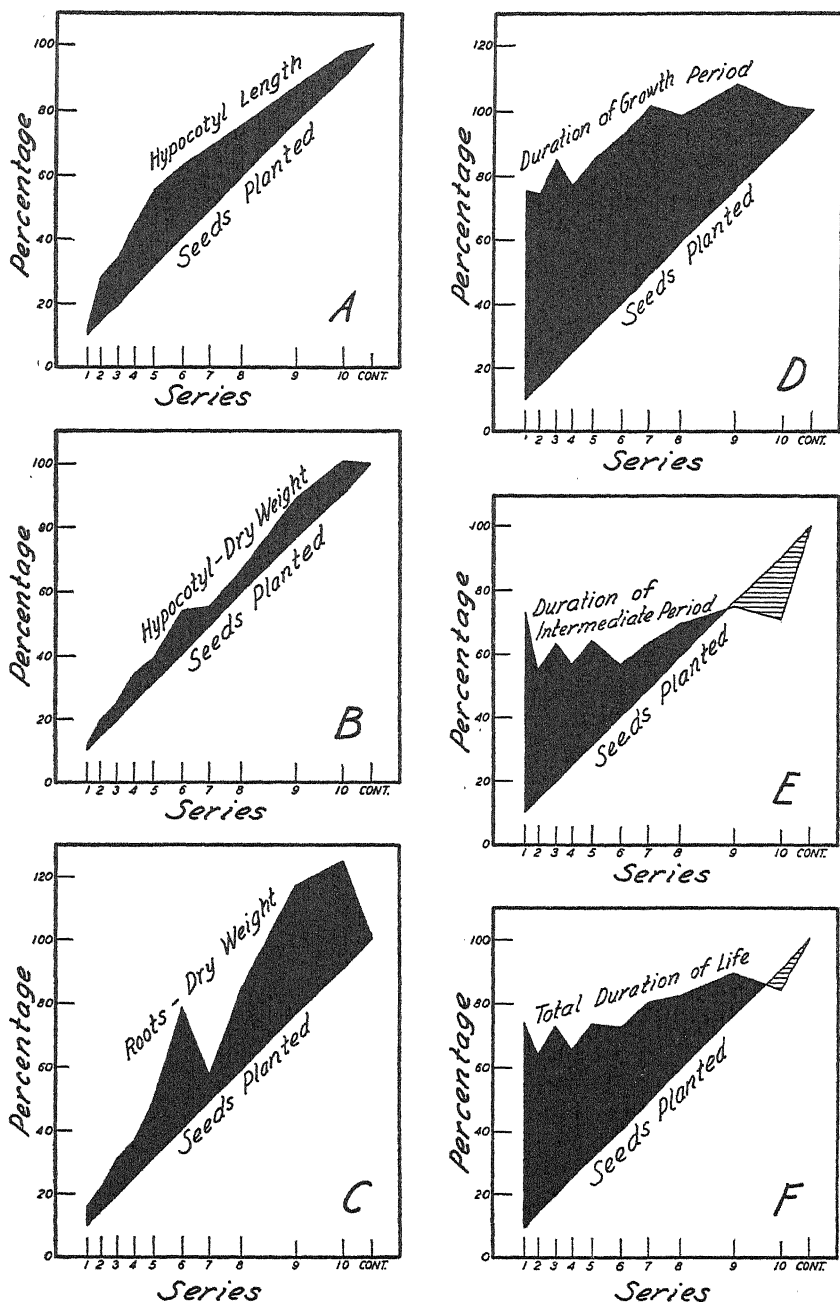


FIG. 4. Diagrams comparing the performance of the seedling plants with the amount of available nourishment (mean weights of planted seeds) in respect of: A, hypocotyl length; B, dry weight of hypocotyl; C, dry weight of roots; D, duration of growth period; E, duration of intermediate period; F, total duration of life. For further explanation see text.

From Fig. 4 and its underlying data the following points may be observed :

1. The performance of the seedlings in respect of length of hypocotyl at the end of the growth period, tends to be relatively more in excess of expectation from the amount of food available, in those series where the larger amounts of the cotyledon had been removed surgically, with the exception only of Series 1. The actual percentage excesses (Column (b)—Column (a), Table II) are as follows: Series 1, +1.9; Series 2, +13.4; Series 4, +19.9; Series 5, +23.9; Series 6, +22.8; Series 7, +19.7; Series 8, +16.4; Series 9, +11.0; Series 10, +7.5. Leaving out of account Series 1, 9, and 10, the hypocotyl lengths are from about 13 to just under 24 per cent. in excess of the values expected from strict proportionality between performance and available food. The greatest excess performance for this variable are found in Series 5 and 6, from which about 69 and 60 per cent. of the cotyledons had been surgically removed.

2. The relative excess in final dry weight of the hypocotyl shows comparatively little variation in the several series after Series 3. The actual percentages of excess performance (Column (c)—Column (a), Table II) are: Series 1, +1.5; Series 2, +4.7; Series 3, +5.4; Series 4, +9.2; Series 5, +7.5; Series 6, +13.7; Series 7, +6.1; Series 8, +7.3; Series 9, +13.0; Series 10, +10.2. There is evidently no close correlation in the performance of the plants between length and final dry weight of the hypocotyl, except in the fact that in all series there is an excess performance in respect of both variables, over the expectation on the basis of strict proportionality to available food.

3. The final dry weights of the roots give a quantitatively different picture (see Fig. 4, C) from the hypocotyl. There is excess performance over expectation, but it is generally much greater in amount than in the case of the hypocotyl. The actual percentages for excess performance in final dry weight of roots (Column (d)—Column (a), Table II) are as follows: Series 1, +6.1; Series 2, +7.4; Series 3, +11.1; Series 4, +11.6; Series 5, +17.6; Series 6, +38.5; Series 7, +7.9; Series 8, +24.1; Series 9, +40.4; Series 10, +34.4. The value for Series 7 seems clearly to be out of line with those on either side of it. We are at a loss to account for this. The separate determinations for each individual seedling are uniformly consistent in showing low values. In general the excess of relative performance over expectation in respect of final dry weight of roots was greatest in the series numbered 6 and above. These were the series from which less than 60 per cent. of the cotyledons (by weight) had been removed. The figures of Tables I and II show plainly that one effect of the operative procedures was to alter the proportions of hypocotyl and roots to each other in the completely grown seedlings. This point will be discussed in a later section.

4. Since there is an excess performance of the operated seeds over expectation on the basis of available food for hypocotyl growth and for root growth, each considered separately, there obviously is bound to be an excess performance in the growth of both organs when taken together, hypocotyl+roots. The actual percentages for excess performance in final dry weights of hypocotyls+roots (Column (e)—Column (a), Table II) are: Series 1, +2.2; Series 2, +5.2; Series 3, +6.4; Series 4, +9.6; Series 5, +9.2; Series 6, +17.9; Series 7, +6.4; Series 8, +10.2; Series 9, +17.5; Series 10, +14.3.

5. In the light of preceding results the final dry weight of the cotyledons in the operated seeds would be expected to show a systematic relative defect from the controls, having regard to the amounts removed in each series by operation, since we are dealing with a closed system in which all the material making up the dry weight of the grown seedling (hypocotyl+roots) was derived by metabolic translocation from the stored food reserves in the cotyledons at the start, and since, as has been shown, the seedlings in all the operated series exhibit an excess performance in final dry weights of hypocotyl+roots. In a general way the dry weights of the cotyledons show the expected result, but not as clearly and regularly as might be desired. The experimental difficulties here are considerable. The cotyledons at the end of the experiment are, in the first place, absolutely small, and, in the second place, difficult to dry accurately to constant weight, owing to their structure and physical consistency. The actual percentage deviations from expectation in this variable (Column (f)—Column (a), Table II) are as follows: Series 1, -0.8; Series 2, +2.7; Series 3, +1.4; Series 4, -4.4; Series 5, -6.7; Series 6, +2.0; Series 7, -1.8; Series 8, +3.2; Series 9, -9.2; Series 10, -0.7. Thus six of the ten operated series show a defect (- sign), and four an excess. The negative (defect) percentages sum to -23.6, while the positive (excess) percentages add to only +9.3.

6. The final dry weights for the whole plant (hypocotyl+roots+cotyledons) show a relative performance in excess of expectation on the basis of available food in each of the operated series, as would be expected from what has preceded.

7. We come now to the consideration of total duration of life of the seedlings, and duration of each of the two principal components of the life cycle, the growth period, and the intermediate period. Considering first the growth period, it is seen from Fig. 4, D that, in all the operated series without exception, this was greatly prolonged beyond expectation, considering the performance of the controls and the amount of food available for the growth of the operated plants. The actual percentage of excess performance in duration of the growth period (Column (h)—Column (a), Table II, are: Series 1, +65.2; Series 2, +59.9; Series 3, +65.2; Series 4,

+51.8; Series 5, +53.7; Series 6, +52.4; Series 7, +52.8; Series 8, +39.5; Series 9, +32.0; Series 10, +10.9. These are high values, and show plainly how great is the relative prolongation of the growth period in the operated plants. It will be further noted that there is a definite trend in the results, indicating that the greatest relative prolongation is in the earlier series, where the greatest amount of cotyledonary material was removed by operation.

8. The intermediate period of the life cycle, between the end of growth and the beginning of disintegration and death, also shows relative prolongation over expectation in the first eight series of operated plants inclusive. In Series 9 and 10 the intermediate period is less long than would be expected considering the performance of the controls and the amount of food available. The actual percentages of excess (or defect) performance (Column (i)—Column (a), Table II) are as follows: Series 1, +63.1; Series 2, +40.5; Series 3, +43.6; Series 4, +31.8; Series 5, +33.2; Series 6, +16.5; Series 7, +14.2; Series 8, +10.1; Series 9, -1.4; Series 10, -19.9. It is evident that the greatest relative prolongation of the intermediate period is in the earlier series, from which the greatest amount of cotyledonary material was removed by operation, just as was the case for the growth period. The amount of this prolongation is large in the first five series grown from operated seeds.

9. The total duration of life, from planting to the beginning of death (growth period + intermediate period) is relatively prolonged over expectation, as necessarily follows from what has preceded, in all the series of plants grown from operated seeds except the last, Series 10. The actual percentages of excess performance (Column (j)—Column (a), Table II) are: Series 1, +64.0; Series 2, +49.1; Series 3, +53.1; Series 4, +40.6; Series 5, +42.2; Series 6, +32.3; Series 7, +31.0; Series 8, +23.0; Series 9, +13.3; Series 10, -6.3.

10. The significance of the results regarding duration of life may be made apparent in another way. If the control seedlings had lived as long in proportion to the food they had available as did the seedlings in Series 1, their mean total duration of life would have been slightly over 6,060 hours instead of the 816 hours which they did live. In other words, the life of the seedlings in Series 1 was prolonged nearly *seven and a half times* more proportionately than that of the normal control seedlings. Shear (26), as already noted on p. 580 *supra*, reports greatly increased duration of life of peanut seedlings, under the conditions of his experiments, from which the cotyledons had been excised.

The more general discussion of the results so far set forth will be deferred to a later section of this paper, after certain other relations have been examined.



*C. Relative Proportions of Hypocotyls and Roots in Seedlings from Operated Seeds.*

In the preceding section it was pointed out that the final dry weight figures indicated that the seedlings from operated seeds had developed a heavier root system in proportion to the hypocotyl, or to the total plant, than had the controls. We may now examine this point in detail. Table III gives the percentages which the final dry weights of the hypocotyl and the roots respectively are of the final dry weights of the whole plant.

TABLE III.

*Percentages of (a) Hypocotyl and (b) Root Final Dry Weights to Final Dry Weight of the Whole Seedling.*

Series.	Hypocotyl. (a)	Roots. (b)
1	57.0	16.3
2	57.1	13.3
3	57.5	14.4
4	63.8	13.9
5	61.6	15.8
6	57.1	17.0
7	58.5	12.3
8	55.5	14.2
9	58.8	15.7
10	56.5	14.3
Controls	56.0	11.4

The figures of Table III bring out what was clear from direct observation of the plants in the experiments as they were growing, namely, that the root systems in the operated series were larger and bore more and longer lateral rootlets than in the controls, or than unoperated canteloup seedlings in other experiments grown under the same conditions. In this respect the operation has had the effect of altering the normal and usual morphological pattern of the seedling.

Table III also brings out another point consilient with the results recorded in the preceding section. Those results indicated that the performance of the operated seedlings in respect of growth was uniformly in excess of expectation on the basis of strict proportionality of available food, in regard to both hypocotyl and roots. This can only mean that the operated seedlings translocated metabolically a larger proportion of the plastic food material of the cotyledons to both hypocotyl and roots than did the normal controls. Table III shows, as it should, that not only do the roots in the operated series exhibit a higher proportion of the final dry weight of the whole plant than in the controls, but also the hypocotyls in

the operated series show small, but consistently (Series 8 forming the only exception) higher percentages of the final dry weight of the whole plant.

This leads us to the consideration of :

*D. The Relative Metabolic Translocation of Material from Planted Seed to Completely Grown Seedling.*

In Table IV are shown the percentages which the final dry weights of hypocotyl+roots are of the weight of the seeds planted, the means of Table I being used for the calculations.

TABLE IV.

Series.	Percentage.
1	48.8
2	53.7
3	52.5
4	55.0
5	51.4
6	57.4
7	44.9
8	46.6
9	48.8
10	46.0
Control	39.7

It will be understood, of course, that the figures of Table IV have no particular absolute significance. Whatever meaning they have derives from their comparative values among themselves. The percentages are computed by the following formula :

$$\frac{100 \text{ (Final dry weight of hypocotyl + roots of seedling)}}{\text{Weight of seed planted}}$$

The weight of the seed planted is made up of two components: (*a*) the embryo, and (*b*) the cotyledons. Furthermore, the weight recorded is a fresh weight and includes the normal water of organization of the ordinary air-dry, ripe, stored seed. So the comparison is obviously defective from the standpoint of absolute values. But it seems probable, as a rough first approximation, that the water in the seeds planted bears a reasonably constant proportion to their total weight, and that the weight of the embryo similarly forms an approximately constant proportion of the total weight of the seed planted.

With these reservations and assumptions in mind it is seen from Table IV that the final dry weight of the grown plant (hypocotyl+roots) bears consistently a higher proportion to the weight of the seed planted in all the operated series than in the controls. The differences range from 5.2

to 17.7, and the consistency of the results is impressive. Furthermore, it is noteworthy that the differences are generally larger in the first six operated series, in each of which more than half of the cotyledons was removed by operation, than in the last four operated series. Shear (26), in his starved peanut seedlings, found evidence of a more efficient use of oil reserves than in the normal seedlings.

From these results it may be tentatively inferred that the operation acted *per se* as a stimulus to the more efficient metabolic transference of plastic food material from the cotyledons to the growing seedlings than that normally occurring in the unoperated plant.

#### E. Some Characteristics of the Growth Curves.

As has already been stated above, the growth curves for elongation of the hypocotyl (Fig. 3) were graduated (by the method described in Pearl (19)) with logistic curves having the general equation

$$y = \frac{K}{1 + Ce^{rx}}$$

In this equation  $y$  = seedling height (length of hypocotyl),  $K$  = the final asymptotic height,  $x$  = age of seedlings, and  $C$  and  $r$  are constants respectively defining the position and steepness (growth rate) of the curves. The simple logistic equation used gives rise to symmetrical curves. While certain of the series could have been more closely fitted by skew logistics (with power terms in the exponent of  $e$ ) the symmetrical fits were adequate for the present purpose, which is to compare the constants  $K$  and  $r$  in the several series.

The values of these two constants are given in Table V.

TABLE V.  
*Constants of Logistics.*

Series.	Mean weight of seeds planted (mg.).	$K$	$r$
1	2.03	1.69	-0.556
2	2.96	3.96	-0.758
3	4.00	4.86	-0.682
4	5.07	6.35	-0.740
5	6.38	7.83	-0.697
6	8.21	8.95	-0.658
7	9.98	9.74	-0.581
8	12.03	10.70	-0.634
9	15.57	12.39	-0.587
10	18.42	13.88	-0.576
Controls	20.37	14.17	-0.587

From the data of Table V we note the following points :

1. The mean asymptotic heights of the hypocotyls in the several series ( $K$ ) increase logarithmically with the mean weight of the seeds

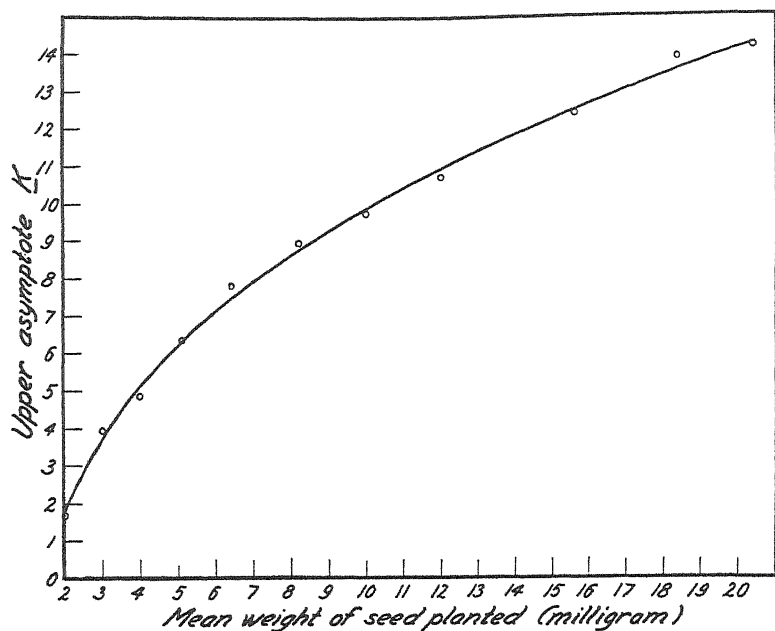


FIG. 5. Values of  $K$  from logistic equations (circles) and fitted curve.

planted, according to the following equation, which was fitted to the data by least squares :

$$K = -1.536 + 0.1097 w + 10.3451 \log_{10} w,$$

where  $w$  is the weight of the seed planted. That the relationship is a close one is clearly shown in Fig. 5.

This curve may be interpreted physiologically to mean that the larger amount of the cotyledons removed by operation the more efficiently the plant transferred plastic material from the stored reserve and laid it down in the growing seedling. In short the curve confirms from another angle the results reached in earlier sections of the paper.

2. The value of  $r$ , which may be taken to measure the inherent growth rate (see, in this connexion, Reed and Pearl (24), and Merrell (14)), decreases arithmetically with increasing weight of seed planted. That is to say, the larger the amount of the cotyledons removed by operation, the more rapid was the inherent growth rate, with Series 1 alone forming any marked exception to this rule. Again we see an apparent stimulating effect of the operation *per se*, this time upon growth rate in time.

3. As a necessary correlate of the result set forth in the preceding

paragraph, the abscissa of the point of inflection—that is, the age of the seedling at the instant when the growth is most rapid per unit of time—increases with the weight of the seed planted. With some fluctuations

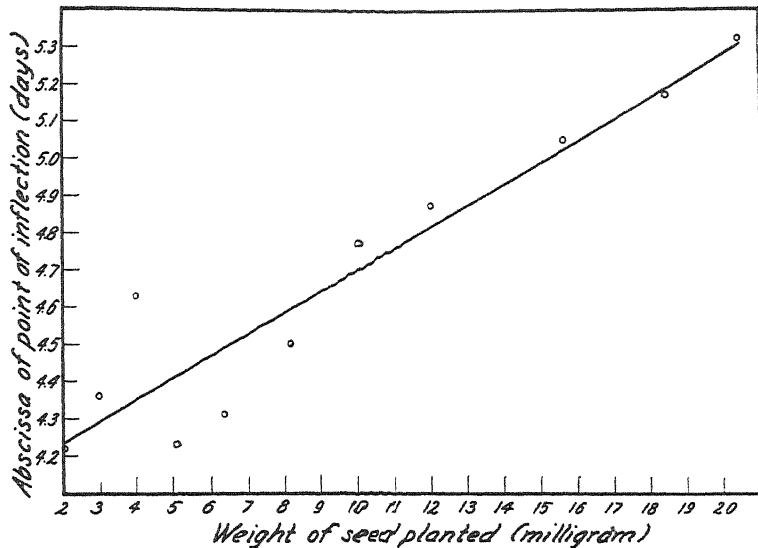


FIG. 6. Abscissae of the point of inflection (circles) of the logistic curves fitted to the several experimental series, and fitted straight lines.

due presumably to experimental and observational errors, the relationship between these two variables appears to be linear, and to follow the equation :

$$I = 4.121 + 0.0580w,$$

where  $I$  denotes the point of inflection (in days of age) and  $w$  is weight of seed planted. Fig. 6 shows the result graphically.

The marked postponement of the period of most rapid growth as smaller and smaller amounts of the cotyledons are removed by operation is apparent, culminating in the control series at the end, which took the longest time of all to attain its maximum rate of growth.

#### IV. DISCUSSION.

The experimental results presented above appear to be clear-cut and consistent. Under experimental conditions so organized that each seedling of *Cucumis melo* has as sources of material and energy for its vital activities only what is stored within the seed from which it is itself derived, plus water and air, it goes through an abbreviated life cycle divisible into three characteristic phases or periods, viz. (a) the growth period, (b) an intermediate period of integrated maintenance of life, and (c) a period of disintegration and death. The seedlings form hypocotyls and roots only,

and the hypocotyl grows in length according to a logistic curve. In the closed system of the experimental conditions, and with the extreme care used to obtain uniformity of conditions, the seedlings are extremely constant, i.e. exhibit little variation among themselves, in respect of all their vital activities, growth, duration of life, &c.

It is shown in the present paper that if, prior to planting and rearing under described conditions, the seeds have different measured amounts of tissue removed under aseptic surgical precautions from the distal ends of the cotyledons, the subsequent performance of the seedlings in respect of both growth (however measured) and duration of life (either in total or in each of its component parts separately) is *not* proportional to the amount of plastic food material available in the cotyledons after operation, but is instead considerably in excess of expectation on the basis of strict proportionality. The observed results warrant the statement that operative procedure has stimulated the seedlings to super-normal performance in growth and longevity.

But to say that the operative procedure has 'stimulated' the seedlings is merely to describe the observed facts in different words. It really tells nothing new or significant about the physiological processes involved. What appears to be the important physiological fact is that in the seedlings derived from operated seeds there is a more extensive and efficient metabolic translocation of plastic food materials from the cotyledons to the plant than occurs in the normal, unmutated individual. Furthermore, within limits, this effect is greater in proportion as the operative removal of cotyledonary tissue is more extensive.

These observations imply, as a corollary, that the seedlings from a normal, unmutated seed do not withdraw from the cotyledons the total amount of available, plastic food material there stored. Instead growth, and later in turn, life itself, come to an end in normal seedlings leaving behind in the cotyledons material for further growth and longer living respectively. In short, in the normal organization pattern of the plant there is an excess of stored plastic material in the cotyledons, constituting what Meltzer (13), in the essay which has become a classic of animal physiology, has called a 'physiological factor of safety'. Meltzer discussed many examples of this in the animal economy, and showed that under conditions of exceptional physiological stress or difficulty the reserves constituting the factors of safety could be, and readily were, drawn upon by the organism. For example, after the removal of one kidney from a normal, healthy animal the amount and composition of the urinary secretion remains practically unaltered, even soon after the operation. That is, the remaining kidney is able to do twice its normal amount of work. The ability of the human organism under exceptional conditions, to utilize its normally untapped reserves has been discussed in a famous essay by William James (10).

Under normal conditions the general integration of the organism as a whole appears to produce an inhibiting effect upon the free and unlimited use of the reserve factors of safety in such processes as growth, for example. If canteloup seedlings are grown under conditions identical with those described in this paper, save in the one respect that they are grown in the light, growth in length of the hypocotyl is much inhibited. That is, of course, a phenomenon well known to all plant physiologists. The alteration of the normal integrated pattern of the plant by the operative procedures used in the present experiments appears to have removed to a considerable degree the usual inhibitions in respect of utilization for growth and duration of life of the stored reserves of food in the cotyledons.

Work along the lines described in this paper is being continued, with the object of further analysing the physiology of the excess relative performance of seedlings from operated seeds.

#### V. SUMMARY.

The principal results of this study may be summarized as follows:

1. Under aseptic precautions portions of the cotyledons of *C. melo* were removed surgically. The amounts of cotyledonary tissue so removed ranged from about 10 per cent. to about 90 per cent. These seeds were then planted and grew in the dark at constant temperature under sterile conditions so arranged that the growing plant was dependent solely upon endogenous nourishment (plus water and air), and their performance in respect of growth and duration of life was compared with that of normal seedlings from unoperated seeds grown under the same conditions.

2. The growth of the hypocotyl in length was in excess of that expected from the available food in all of the series from operated seeds, by amounts ranging from approximately 2 per cent. to 24 per cent.

3. The same relative excess performance over expectation from proportionality to available food was also exhibited in all the series from operated seeds, in respect of final dry weight of the hypocotyl (by amounts ranging from 1.5 per cent. to 13.7 per cent.); in the final dry weight of the roots (by amounts ranging from 6.1 per cent. to 40.4 per cent.); in the hypocotyl + roots; and in the whole plant, hypocotyl + roots + cotyledons.

4. The same relative excess performance following operation was also found in respect of the duration of the growth period (by amounts ranging from 10.9 per cent. to 65.2 per cent.); in the duration of the intermediate period of the life cycle, except in the two series from which the smallest and next smallest amounts of cotyledonary tissue had been removed (by amounts ranging from 10.1 per cent. to 63.1 per cent.); and in the total duration of life of the plant, except in the one series from which the

smallest amount of cotyledonary tissue had been removed (by amounts ranging from 13.3 per cent. to 64 per cent.).

5. The root system made up a higher proportion than normal in the seedlings grown from operated seeds.

6. The final dry weight of hypocotyl + roots bore a higher proportion to the initial weight of the seed planted in the seedlings from operated seeds than in the normal controls, whence it is inferred that the metabolic translocation of reserve food material from the cotyledons to the growing plant was more efficient than in the normal unaltered seedling.

7. The larger the amount of cotyledonary tissue removed by operation the more rapid was the inherent growth rate of the seedling per unit of time.

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# The Chromosomal Relationships in the Swede and Turnip Groups of Brassica.

BY

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With seventy-two Figures and two Diagrams in the Text.

## INTRODUCTION.

*BRASSICA napus* L. (swede) and *B. rapa* L. (turnip), as defined by Hegi (11), are species that include a wide range of forms, exhibiting very often parallel modifications of habit and structure. Within each natural group the forms freely intercross, giving fertile hybrids, but crosses between forms one from each group are more difficult of attainment, and the  $F_1$  hybrid is correspondingly more sterile.

A cytological study of these two groups was undertaken to elucidate certain genetical, cytological, and agricultural problems. These are respectively (1) the relationship of swede, swede-like rape, and bulbless bolter (a rogue appearing in crops); (2) the truth of assertions that certain turnips (Fosterton Hybrid and The Bruce) have swede ancestry; (3) the possibility of combining swede and turnip by hybridization to produce a new amphidiploid species (actually accomplished by Frandsen and Wage, 1932 (7)); (4) the reason for duplicated factors governing certain swede characters and the similar occurrence of multiple factors in *B. oleracea*; (5) the reason for the different basic numbers shown by the two groups. Our previous knowledge is dealt with in the discussion in relation to the new facts brought out by this study.

The study was commenced in the Department of Botany at the University of Glasgow in 1929, at the suggestion of Professor J. M. F. Drummond, and completed at the University of London, King's College, during 1931.

## MATERIALS AND METHODS.

Almost all of the seeds and plants used were obtained from Dr. V. McM. Davey of the Scottish Society for Research in Plant-Breeding at Corstorphine.

They consisted chiefly of pure lines or pedigree crosses made by him, together with a few seeds direct from commercial sources. Three half-bulbed bolters, from a swede crop, were received from Dr. D. G. O'Brien of the Agricultural College, Glasgow, through the agency of Professor Drummond; two were examined cytologically and one was sent to Corstorphine for use in genetical studies. They proved to be swede by swede-like rape hybrids.

Root-tip material was obtained by germinating seeds on damp filter paper in Petri dishes. Germination is rapid and tips are ready in two or three days. Fixations were made with Bouin, Carnoy, Navashin, and various modifications of Flemming's fluid, including 2 B (La Cour, 1931 (17)). A Flemming modification, rather like that recommended by Taylor for smears (see McClung, 1930 (26)), proved very useful. Its formula is:

osmic acid 2 per cent.	1.5 c.c.
chromic acid 10 per cent.	3 c.c.
acetic acid 10 per cent.	2 c.c.
water	10 c.c.
maltose	ca. 0.2 gram.

Penetration is rapid and the general fixation is perfect; somatic metaphase plates show the chromosomes well spaced and without any sign of clumping. The root-tips were cut at  $15\mu$ .

Preliminary inspections of meiosis and also counts of chromosomes were made from mounts in Belling's iron aceto-carmine fluid. The details were studied exclusively in permanent smears fixed in strong, medium, or a modified Flemming fluid.

All permanent preparations were stained with iodine gentian violet, Newton's technique being followed.

#### NUMBER AND MORPHOLOGY OF THE SOMATIC CHROMOSOMES.

Table I summarizes the results for all the forms, except the inter-group hybrids; the list emphasizes the relationship shown between the members within each of the main series and confirms Karpechenko's (1922) (14) counts in similar forms. In particular the numbers show that The Bruce and the Fosterton Hybrid turnip are true turnips. Both these have been popularly supposed to be derivatives of a turnip by swede hybrid. Findlay (1931) (6) believes there may be some basis for this idea in the case of The Bruce, but the cytology is incompatible with this particular hybrid ancestry.

An inspection of Figs. 1-10 (swede group) and of 11-17 (turnip group) will demonstrate also the very close morphological agreement existing between the chromosomes of members of the same group. The attachment constrictions can be made out in all the chromosomes except

TABLE I.  
*List of Chromosome Numbers.*

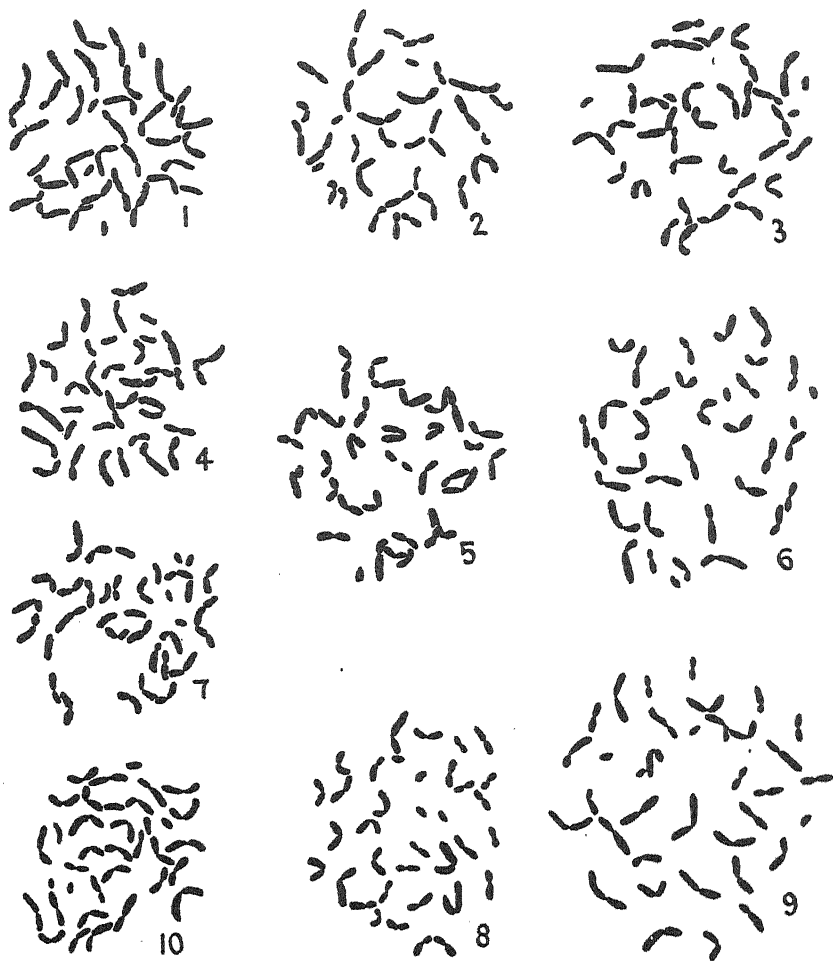
	Variety.	Somatic number.	No. of bivalents at meiosis.
<i>Swede Group.</i>			
Swedes : yellow flesh	Improved purple top	36	—
	Mervue	36	18
	Superlative	36	18
	Improved tankard brown top	36	18
	Darlington	36	18
	Improved green top	36	18
		36	18
white flesh Bulbless bolters	BObba	36	18
	BPa	36	—
	KB	—	18
	Dr. O'Brien's bolters from commercial stock	—	18
		36	18
Winter swede rape		36	—
Rape kale		36	—
Hybrids	swede × bulbless bolter (extracted form)	36	—
	swede × swede rape $F_1$	—	18
	swede rape × swede $F_1$	—	18
	swede rape × bolter (BO) $F_1$	—	18
	swede × bolter (BO) $F_1$	—	18
<i>Turnip Group.</i>			
Turnips	Lincolnshire red globe, white flesh	20	10
	Centenary green top, yellow flesh	20	10
	The Bruce	20	—
	Fosterton Hybrid turnip	20	—
Turnip rapes	Winterrübsen	20	—
	Sommerrübsen	20	—

a few very tiny ones, in which it may be terminal. No supernumerary constrictions or trabants have been observed. Similar chromosome types may be observed in members of the swede and the turnip alliance; but the frequency of the types is different. The resemblances and differences may be summarized as follows:

	Swede group	Turnip group.
Somatic number (diploid)	36	20
Short ( $0.5 \mu$ ) chromosomes	4	2
Long ( $2 \mu$ ) chromosomes, with nearly median attachment	4	2
Medium-length ( $1-1.8 \mu$ ) chromosomes, with median attachment	16	10
Medium-length ( $1-1.8 \mu$ ) chromosomes, with subterminal attachment	12	6

If the swede be regarded as a numerically tetraploid form compared with the diploid *B. oleracea* ( $2n = 18$ ), the turnip may be looked upon as a diploid, tetrasomic in respect of one chromosome type. A hybrid between the two, on the supposition that both regularly form bivalents, may be expected to show ten bivalents and eight univalents, as the minimum

possible association; it is not improbable, however, that trivalents would be formed in greater or less degree.



FIGS. 1-10. Somatic chromosomes in polar metaphase. Fig. 1, purple top swede (IPaan); fig. 2, superlative swede, soft purple top (SUBada); fig. 3, Tankard bronze top swede (ITabca); fig. 4, Darlington, swede, light bronze globe (DLaa); fig. 5, green top swede (IGAabc); fig. 6, white flesh swede (WFba); fig. 7, bulbless bolter (BOBba); fig. 8, bulbless bolter (BPa); fig. 9, winter swede rape (Df3); fig. 10,  $F_2$  of the hybrid, swede  $\times$  bulbless bolter (BOb). All  $\times 3,300'$

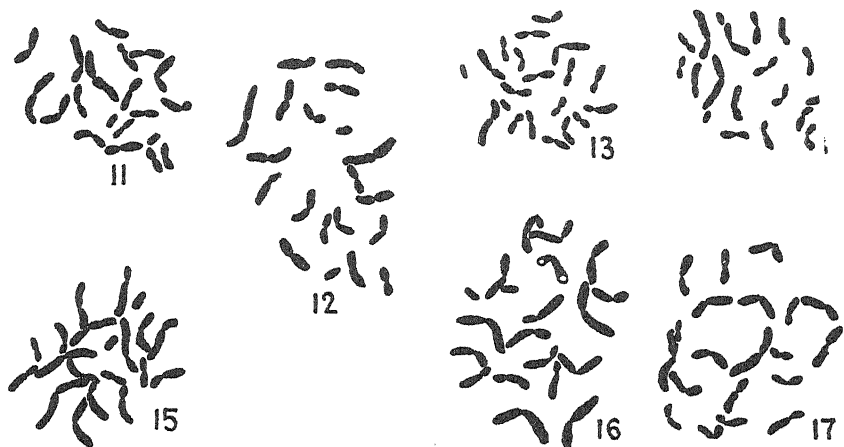
#### MEIOSIS.

##### *Swede Group.*

All the members of the group (swede strains, rapes, and bulbless bolters, together with the hybrids between them) are remarkably alike in the closest details of meiosis. At diakinesis, except in rare instances, there are eighteen bivalents in the nucleus, together with the nucleolus (Figs. 18-

*Swede and Turnip Groups of Brassica.*

20). The forms of the bivalents are various, and regular size differ similar to the differences shown in the somatic chromosomes, may be



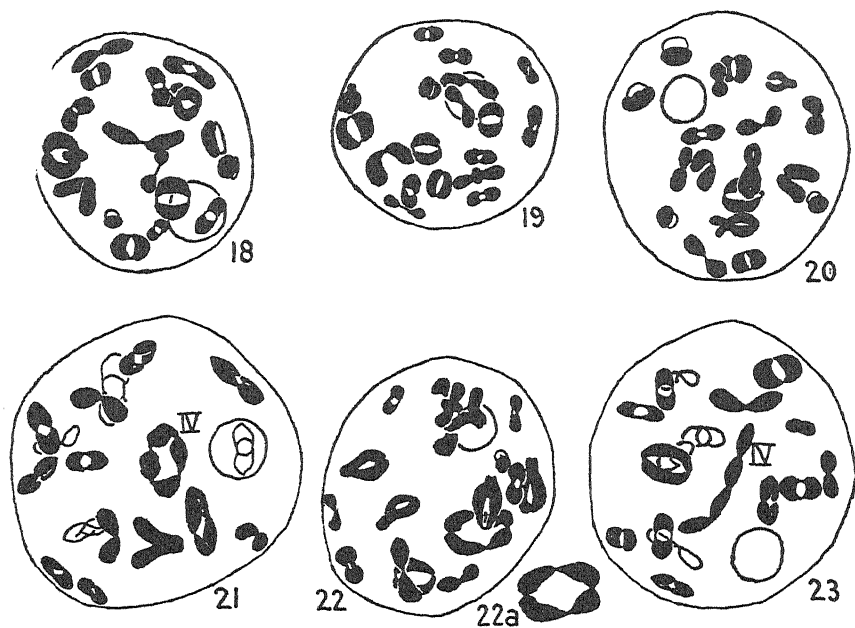
FIGS. 11-16. Somatic chromosomes in polar metaphase. Fig. 11, winter turnip rape (Winterrübsen); fig. 12, summer turnip rape (Sommerrübsen); fig. 13, Lincolnshire red globe turnip; fig. 14, Centenary green-top, yellow flesh turnip; fig. 15, The Bruce; fig. 16, Fosterton Hybrid turnip (ex Bell); fig. 17, Fosterton Hybrid turnip (ex Sharpe). Figs. 11-14,  $\times 3,300$ ; figs. 15-17,  $\times 4,000$ .

The chromosomes at the earlier stages of prophase are too small for study and, moreover, do not fix particularly well. It is evident, however, from their later history that chiasmata are established interstitially and undergo terminal movement. Opening out of the loop containing the attachment constrictions is usually carried to its extreme limit, with the result that the two chromosomes of a bivalent are held together by terminal chiasmata, either one or two in number. Hence, both rod and ring bivalents result, the number of the former being about 5 to 7 or 8, and the latter some 10 or 11 to 13. But not all of the chiasmata terminalize fully, and they are therefore more or less interstitial at diakinesis and metaphase I. When the chiasma is but slightly interstitial, the distal portion of the chromosome is very minute; its position is then difficult to demonstrate directly, but may be inferred from the slight lagging shown at anaphase (Fig. 43). More markedly interstitial chiasmata are shown in several instances in the drawings of diakinesis (Figs. 18-23) and anaphase (Figs. 46-8). Finally, it must be noted that the bivalents are uniformly spaced out at mid-diakinesis and that there is no sign of any mutual attraction between them. Exceptionally, chain or ring quadrivalents or trivalents or univalents may be present; these will be dealt with later on.

At the first division metaphase, a variable number of bivalents are secondarily paired. Secondary pairing may be described as pairing at metaphase resulting from a generalized attraction between bivalents related phylogenetically, though distantly. It is therefore characteristic of

*Catcheside.—The Chromosomal Relationships in the*

oids, and in many cases can be used as a measure of quantitative and ps structural changes in the complement of chromosomes. Bivalents



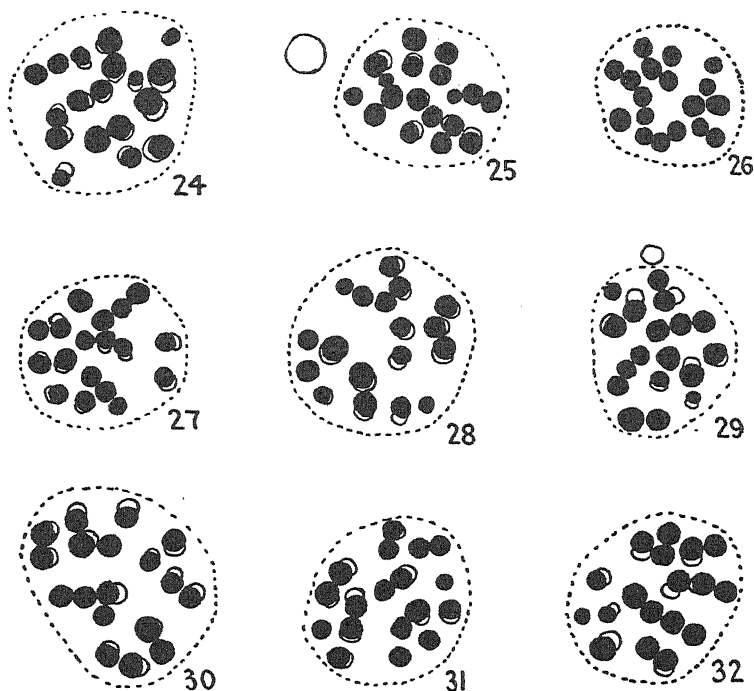
FIGS. 18-23. Pollen mother-cell nuclei at diakinesis. Fig. 18, improved Tankard bronze-top swede (ITabca) showing 18 bivalents; fig. 19, bulbless bolter (BObba) showing 18 bivalents; fig. 20, half-bulbed bolter from commercial crop (ex O'Brien) showing 18 bivalents; fig. 21, improved green-top swede (IGaaba) showing 18 bivalents; fig. 22, winter swede rape (Df<sub>3</sub>) showing ring quadrivalents (also 22a) and 16 bivalents; fig. 23, winter swede rape (Df<sub>3</sub>) showing chain quadrivalent and 16 bivalents. Figs. 18-20 and 22,  $\times 3,300$ ; figs. 21, 22a, and 23,  $\times 4,000$ .

are attracted mutually, but there are no connexions between them and they never appear to touch. The proper orientation of the bivalents on the spindle and their disjunction are unaffected. The secondary pairing may, and often does, persist until division II of meiosis. It has no counterpart at diakinesis when all the bivalents are mutually repelled.

When the two chromosomes of a bivalent are held together at diakinesis and metaphase I by chiasmata, and each chromatid is condensed into a spiral coil, their particulate attractions which led to their pairing at zygotene are released. Prior to condensation, the particulate attractions are internally compensated in pairs, but subsequently many of them are separated from one another and their attractions are released. They may then be exercised on any similar particles within their spheres of influence. But the attachment constrictions appear to be the seat of localized forces leading to their mutual repulsion. The attractive forces appear to diminish more rapidly with increasing distance from a particular chromosome than does the repulsive force. Hence, the sphere of attraction appears to be relatively limited, and it can only be effective when the bivalents are more



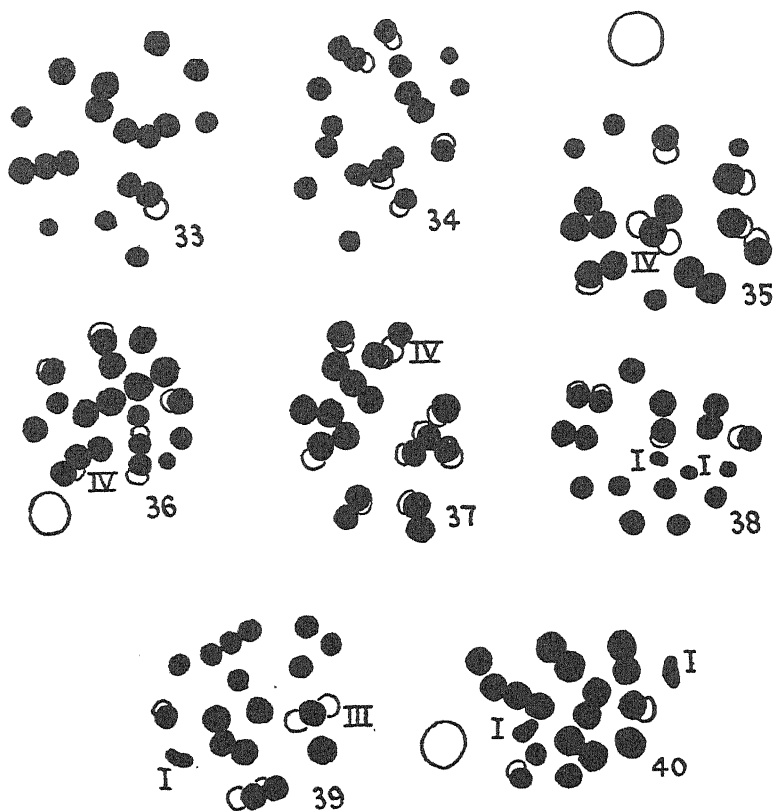
or less closely approximated. This condition is fulfilled at prometaphase, a brief stage between diakinesis and metaphase I, when the bivalents are massed together into a close group. The actual response must depend



FIGS. 24-32. Pollen mother-cells showing polar metaphase I plates all with 18 bivalents having varying degrees of secondary pairing. Fig. 24, Mervue swede (MVabba) showing  $3(1)+4(2)+1(3)+1(4)$ ; fig. 25, improved Tankard bronze-top swede (ITabca) showing  $2(1)+2(2)+2(3)+1(4)$ ; fig. 26, bulbless bolter (BObba) showing  $4(1)+2(2)+2(3)+1(4)$ ; fig. 27, O'Brien half-bulbed bolter showing  $4(1)+2(2)+2(3)+1(4)$ ; fig. 28, winter swede rape (Df3) showing  $3(1)+3(2)+3(3)$ ; fig. 29, swede  $\times$  swede rape  $F_1$  (ABO) showing  $3(1)+3(2)+3(3)$ ; fig. 30, swede rape  $\times$  swede  $F_1$  (ABI) showing  $3(1)+4(2)+1(3)+1(4)$ ; fig. 31, swede  $\times$  bulbless bolter (BObo) (ABF) showing  $6(1)+6(2)$ ; fig. 32, white flesh swede (WFba) showing  $4(1)+2(2)+2(3)+1(4)$ . All  $\times 3,300$ . Figs. 25 and 29 with persistent nucleolus present in cytoplasm.

also upon size, smaller bivalents being moved more easily than larger ones, since it is not found in polyploids with large chromosomes. Subsequently to the prometaphase contraction stage the unpaired bivalents and groups of secondarily paired bivalents repel one another mutually so that they become more or less isolated on the metaphase plate, the secondarily paired bivalents remaining in close association. The mechanism observed by Lawrence (1931) (21) in *Dahlia* has therefore been found to hold for *Brassica*; it has been seen in both swedes and turnips. Since the appearance of secondary pairing provides a test of distant relationship between chromosomes, it will be used here in determining the basic composition of the chromosome complements.

A considerable amount of secondary pairing is found in the swede and its relatives, and it is significant that the multivalent associations of chromosomes thus formed always consist of bivalents of roughly the same size in



FIGS. 33-40. Pollen mother-cells showing polar metaphase I plates illustrating primary and secondary pairing. Fig. 33, O'Brien's half-bulbed bolter showing 18 bivalents associated into  $8(1) + 2(2) + 2(3)$ ; fig. 34, swede rape  $\times$  swede (ABI) showing 16 bivalents as  $9(1) + 3(2) + 1(3)$ ; fig. 35, improved Tankard bronze top swede (ITabca) showing 1 quadrivalent and 16 bivalents, 1 secondarily paired with the quadrivalent and the remainder into the groups  $6(1) + 3(2) + 1(3)$ ; fig. 36, O'Brien's half-bulbed bolter showing 1 quadrivalent and 16 bivalents associated into  $7(1) + 1(1) + 1(3) + 1(4)$ ; fig. 37, improved green-top swede (IGaaba) showing 1 quadrivalent and 16 bivalents with maximum association, viz.  $3(2) + 3(4)$ ; fig. 38, bulbless bolter (KB) showing 2 univalents and 17 bivalents with very little secondary pairing, viz.  $9(1) + 4(2)$ ; fig. 39, bulbless bolter (KB) showing 1 trivalent, 1 univalent, and 16 bivalents, secondarily paired into  $8(1) + 1(2) + 2(3)$ ; fig. 40, improved Tankard bronze-top swede (ITabca) with 2 univalents and 17 bivalents associated into  $5(1) + 4(2) + 1(4)$ . Figs. 35, 36, and 40 with nucleolus in cytoplasm. All  $\times 4,000$ .

all cases where the size differences can be made out in the metaphase plates. In the case of profile views where size differences are perhaps more obvious, the pairing of bivalents almost invariably of similar size is quite clear (Figs. 41-2); moreover, the paired bivalents in any one group show similar chiasmata, either two or one, and usually terminal (see below). Associations between 2, 3, and 4 bivalents have been observed, but rarely

between any higher number, though a considerable number of cells were examined. Apparent associations of more than four chromosomes occur occasionally and may indicate structural complexity in the chromosome complement. Tables II, III, and IV summarize the principal points relative to secondary pairing. Figs. 24-40 and 50 illustrate various types of secondary pairing. Fig. 49 shows a tetraploid cell found in white flesh swede and seen in polar view; it has 36 bivalents with a high degree of (apparently) secondary association.

The number of secondary associations per metaphase plate ranges between 4 and 12, the mode being 9 and the average 8.5 (cf. Table II and Diagram 1). The conditions at the first division metaphase are consistent with the view that the swede is trebly octasomic and trebly tetrasomic, a composition allowing twelve secondary associations as a maximum. The maximum has been seen once only (Fig. 37) in 138 plates; it gives six groups of chromosomes, and 6 is therefore the primary chromosome number.

TABLE II.

Plant variety.	Number of polar first division metaphase plates possessing the following members of secondary associations.								
	4	5	6	7	8	9	10	11	12
<i>Swedes.</i>									
Mervue	—	—	—	—	—	1	—	—	—
Superlative	—	—	—	1	1	1	—	—	—
Improved tankard B.T.	—	1	2	2	3	2	3	1	—
Improved G.T.	—	1	1	3	6	16	6	—	1
white flesh	—	—	—	—	3	6	—	1	—
Total	—	2	3	6	13	26	9	2	1
<i>Bolters.</i>									
BO	—	—	—	—	—	1	—	—	—
KB	1	—	1	3	7	4	—	—	—
O'Brien's	—	—	1	2	2	5	3	1	—
Total	1	—	2	5	9	10	3	1	—
<i>Swede rapes.</i>									
winter	—	—	—	1	1	2	—	—	—
<i>Hybrids.</i>									
swede × swede rape	—	—	—	1	2	3	3	2	—
swede rape × swede	—	1	—	2	4	9	2	—	—
swede × bulbless bolter (BO)	—	—	1	—	4	5	2	—	—
Total	—	1	1	3	10	17	7	2	—
Grand Total 138	1	3	6	15	33	55	19	5	1

The haploid chromosome set may then be represented as follows:

A A A A  
 B B B B  
 C C C C  
 D D  
 E E  
 F F

Such a constitution provides ample basis for the multiple factors known in certain species of Brassicas. Clearly, in the evolution of the swede (and perhaps in other species of *Brassica*) there has been a change in chromosomal balance similar to that found in *Pyrus* (Darlington and Moffett, 1930 (4)) and in *Dahlia Merckii* (Lawrence, 1929 (19)). Undoubtedly, such changes are of considerable evolutionary significance, though their precise importance cannot be estimated as yet.

TABLE III.

*Types of Secondary Associations at Meiosis in Swede Group. (a) Cases with Eighteen Bivalents.*

Numbers of secondary associations.	Numbers of bivalents in association.				Number of cases.	Totals.
	1	2	3	4		
4	10	4	—	—	1	1
5	9	3	1	—	1	2
	10	2	—	1	1	
6	6	3	2	—	1	5
	6	4	—	1	1	
	6	6	—	—	1	
	9	1	1	1	1	
	8	2	2	—	1	
7	5	5	1	—	1	4
	4	7	—	—	1	
	6	3	2	—	1	
	7	2	1	1	1	
8	5	3	1	1	7	24
	3	6	1	—	4	
	3	4	1	1	1	
	4	4	2	—	6	
	5	2	3	—	4	
	6	1	2	1	1	
	4	5	—	1	1	
9	3	4	1	1	8	49
	3	3	3	—	20	
	4	2	2	1	8	
	2	5	2	—	8	
	4	3	—	2	1	
	5	1	1	2	4	
10	2	3	2	1	10	16
	1	4	3	—	1	
	3	2	1	2	2	
	1	5	1	1	3	
11	1	3	1	2	4	5
	2	2	—	3	1	
12	—	3	—	3	1	1
						107

Table III summarizes the different types of secondary association, together with the frequency of each. Cells with univalents and other

atypical associations are omitted from the total of 107 plates noted. It is clear that the modal frequency is 9, i.e. that three-quarters of the possible secondary associations are most frequently developed. In Table IV, which summarizes the results for those plates (16 in number) which have univalents or, in one case, a trivalent and a univalent (Fig. 39), the modal frequency of secondary associations is 8, one less than in the case of plates having complete bivalent formation.

TABLE IV.

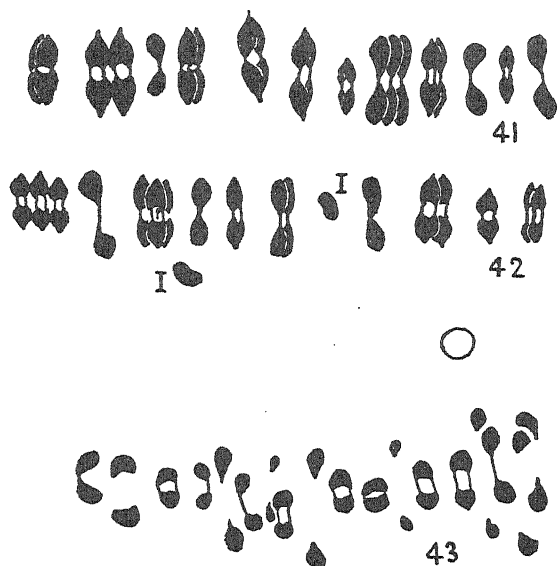
*Types of Secondary Association at Meiosis in Swede Group. (b) Cases with Univalents, &c.*

Numbers of secondary associations.	Number of bivalents in association.				Number of univalents.	Number of trivalents.	Cases.	Totals.
	1	2	3	4				
5	7	5	—	—	2		1	1
6	5	6	—	—	2		1	2
	7	2	2	—	2		1	
7	5	4	—	1	2		2	4
	5	1	3	—	1	1	1	
	6	2	1	1	2		1	
8	3	4	2	—	2		4	6
	4	3	1	1	2		1	
	5	1	2	1	2		1	
9	3	2	2	1	2		1	3
	2	3	3	—	2		1	
	3	3	—	2	2		1	
								16

It is difficult to analyse fully the whole complement in any one cell as seen in profile at metaphase, owing to the secondarily paired groups lying obliquely or perpendicularly to the plane of the microscope field. But, in cases where the number of secondary associations is relatively low, observation is materially improved and the nature of the pairing susceptible of interpretation. In favourable cases (Figs. 41 and 42) the two chromosomes of the bivalents may be seen held together by terminal chiasmata at one or both ends and ranged closely by the side of one or more other bivalents of a like size and, usually, construction. The secondary pairing may involve both pairs of chromosomes of the bivalents each to each, or be restricted to one of them; the former condition is much the more usual.

The close relationship between swedes, swede-like rapes, and bulbless bolters is emphasized by the conditions at meiosis. (1) All three groups of plants, together with their  $F_1$  hybrids, show similar degrees of secondary pairing, for the modal frequency, nine, is the same in each group. There is every reason to suppose that the different forms have identical haploid

sets. (2) The different groups of forms and their  $F_1$  hybrids most frequently form 18 bivalents at meiosis. (3) The frequency of formation of



FIGS. 41-3. Pollen mother-cells at metaphase I and early anaphase as seen in profile; N.B. chromosomes and chromosome-groups separated laterally. Fig. 41, improved green-top swede (IGAaba) metaphase I with 18 bivalents associated secondarily into  $7(1) + 4(2) + 1(3)$ ; fig. 42, improved green-top swede (IGAabc) metaphase I with 2 univalents and 17 bivalents secondarily associated into  $5(1) + 3(2) + 2(3)$ ; fig. 43, Mervue swede (MVabba) anaphase I with 18 bivalents disjoining—note lagging of some bivalents and extra spindle nucleolus. All  $\times 4,000$ .

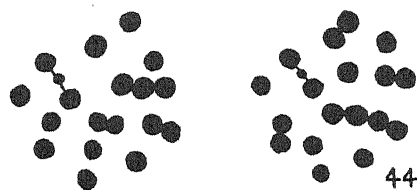


FIG. 44. Swede  $\times$  bulbless bolter (BOba)  $F_1$  (ABF) pollen-mother cell at early anaphase I showing persistent secondary pairing. The left-hand figure represents the upper focus, the right-hand the lower focus; the natural arrangement may be regained by superimposing the two figures. The group at 10 o'clock presumably represents a quadrivalent (*vide* text).  $\times 3,300$ . Fig. 44a is a diagrammatic representation of the prior metaphase condition of the group as seen in profile.

univalents and multivalents is no higher in the  $F_1$  hybrids than in the pure races. (4) The frequency of chiasmata at mid-diakinesis is substantially the same in all the forms examined, and is not reduced in their hybrids.

It has not been possible to make extensive counts of whole nuclei, Table V embodying only nuclei fully interpreted.

Anaphase proceeds normally, there being no appreciable lagging or precocity of separation due to the superimposed secondary pairing. The secondary associations thus interfere neither with normal disjunction of the members of a bivalent nor, presumably, with the randomness of the assortment in respect of all the bivalents. During anaphase, the secondary associations persist and observation of such stages is most instructive in demonstrating the nature of the associations at metaphase. Fig. 44 shows two anaphase groups from the same cell, and at Fig. 44 a is a reconstruction of the metaphase condition as it would be seen in profile; the bivalents are shown arbitrarily as involving but a single chiasma each. One group is evidently a true quadrivalent, involving a multiple chiasma; no other example of this has been seen. After anaphase I, secondary pairing mostly disappears; but quite marked cases of it may be observed at the metaphase II (Fig. 45). Lagging at anaphase I, due to interstitial chiasmata, is fairly frequent; different stages are shown in Figs. 46-8.

TABLE V.

*Chiasma Numbers in Swede Group.*

Numbers.	27	28	29	30	31	32	33	Average.
Swede	1	—	2	4	—	1	—	30
Swede rape	—	—	1	1	—	1	—	30
Bulbless bolter	—	—	—	2	—	3	—	31
Swede × swede rape $F_1$	—	—	—	—	—	—	1	33
Swede rape × bolter $F_1$	—	—	—	—	1	—	—	31
Swede × bolter $F_1$	—	—	—	—	—	1	—	32

Certain aberrations call for special attention. It has been noted above that pairing at diakinesis and metaphase I is conditioned by more or less terminal chiasmata; failure to form any chiasmata between the chromatids of the two associated chromosomes results in the formation of univalents. The presence of two such univalents at metaphase has been noted in 15 out of 138 polar metaphase plates examined; examples are shown in Figs. 38 and 40. Univalents are perhaps most frequently formed by the smaller chromosomes and by those with a subterminal attachment constriction. The presence of univalents need not mean deficient gametes, provided that at division I they go at random to opposite poles of the spindle and are not omitted from the nuclei at interkinesis. Two univalents have a 50 per cent. chance of passing to opposite poles.

The rare formation of quadrivalents and other multivalents in a polyploid is a different question. It is most easily explicable if it be supposed that the duplicated chromosomes have become structurally dissimilar by

linear rearrangement of their parts. Structurally altered chromosomes of the same ultimate origin will then be homologous continuously only over short lengths and therefore unable to pair regularly when in competition

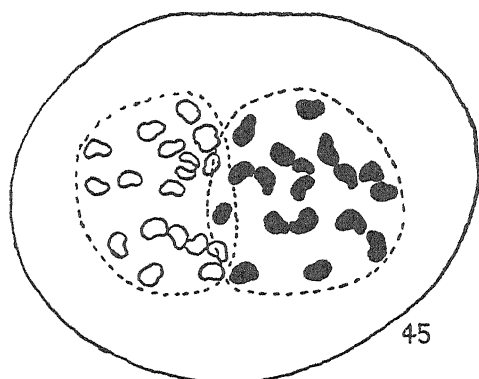
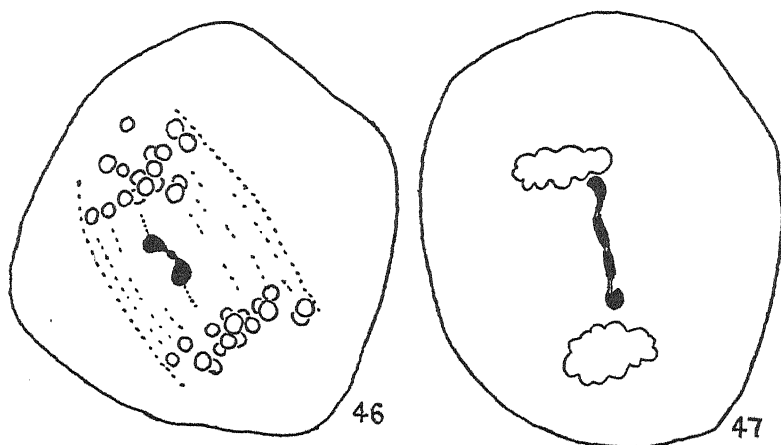


FIG. 45. Improved green-top swede (IGAaba) pollen mother-cell at metaphase II, following a regular 18 + 18 anaphase I disjunction. Note persistence of secondary pairing.  $\times 4,000$ .



FIGS. 46-7. Pollen mother-cells showing bivalents lagging as the result of the presence of interstitial chiasmata in them. Fig. 46, improved green-top swede (IGAaba); fig. 47, superlative swede (SUBada).  $\times 3,300$ .

with unaltered chromosomes. Occasional pairing and chiasma formation would result in multivalents. Diakinesis provides the clearest cases, the commonest multivalent configurations being the ring and the chain of 4 chromosomes (Figs. 21-3). These have also been traced in profile metaphase, though with difficulty, and in polar metaphase (Figs. 35-7, 50). The frequency of occurrence is very low, quadrivalents being found in less than 2 per cent. of cells at diakinesis; exact statistics are difficult to obtain since it is easier to observe a quadrivalent at diakinesis than to count 18 bivalents and so prove its absence.



The behaviour of the nucleolus is noteworthy. Most frequently it does not disappear at the end of the prophase stages and is persistent in a large proportion of cases until the telophase of the second division (cf. Figs. 25, 29, 35, 36, 40, 43; also Figs. 61, 67, 70-2). Its subsequent history is obscure, as it seems to disappear quite suddenly about this time and is not seen again. Fragments also often occur in place of the single large body. The irregular behaviour suggests its comparative unimportance in the economy of the cell.

Pollen grains of most forms were examined in a drop of lactophenol containing cotton blue; they proved to be nearly 100 per cent. good in all cases, a result to be expected in view of the rarity of meiotic abnormalities.

### *Turnip Group.*

The material studied was derived solely from plants of 'Centenary', the green skin, yellow flesh turnip. At diakinesis there are ten bivalents uniformly distributed in the nucleus (Fig. 51). They show size variations, correlated with those found in somatic plates. At metaphase I the bivalents are orientated quite regularly at the equator and show frequent secondary pairing. The extent of this as seen in polar view (Figs. 52-8) is summarized in Table VI and shown pictorially in Diagram 1. The various types of secondary association are shown in Table VII. In profile

TABLE VI.

### *Secondary Associations in Turnip.*

Number of secondary associations per plate	—	1	2	3	4
Number of plates (total 35)	—	3	10	16	6

TABLE VII.

### *Types of Secondary Associations in Turnip.*

Number of secondary associations.	Number of bivalents per association.			Number of cases.	Total.
	1	2	3		
1	8	1	—	3	3
2	6	2	—	7	10
	7	—	1	3	
3	4	3	—	8	16
	5	1	1	8	
4	2	4	—	2	6
	3	2	1	3	
	4	—	2	1	

metaphase I (Fig. 59) secondary pairing is seen to be between morphologically similar bivalents; three bivalents have each a single terminal

chiasma, the other seven two each. Not more than four associations could be observed definitely in any cell, though a large number of cells have been examined. The type of association at this apparent maximum is

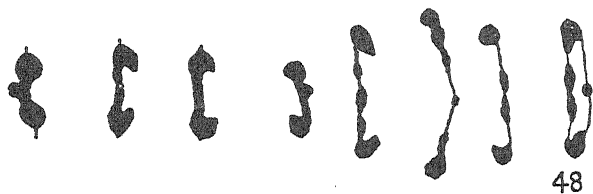
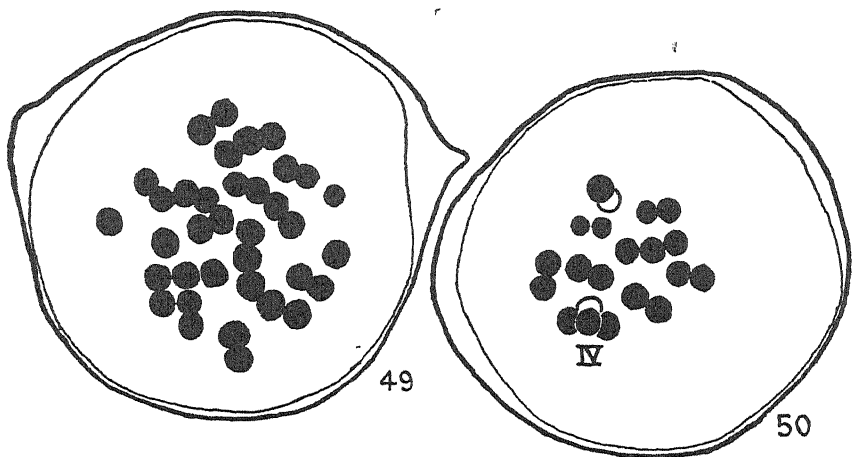


FIG. 48. Superlative swede (SUBada), lagging bivalents selected from eight different cells showing various interstitial chiasmata and stages in their separation at anaphase I.  $\times 3,300$ .

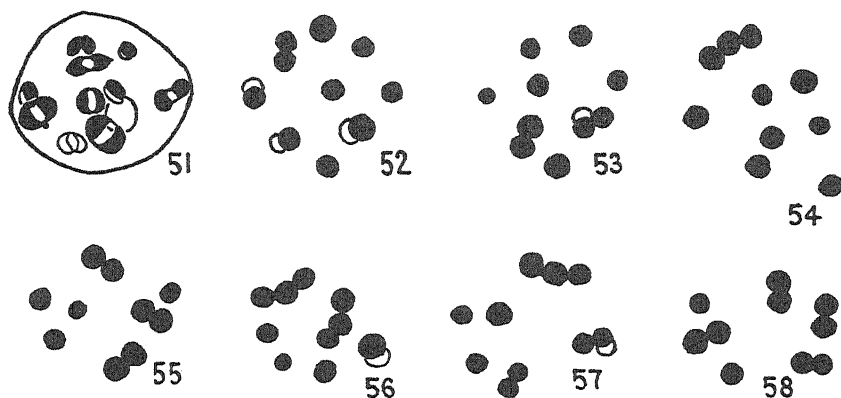


FIGS. 49-50. White flesh swede (WFba) pollen mother-cells at metaphase I in polar view. Fig. 49, tetraploid cell with apparently 36 bivalents showing a high degree of secondary pairing (see text); fig. 50, a diploid cell from same preparation, showing 1 quadrivalent and 16 bivalents secondarily associated into  $1(1) + 6(2) + 1(3)$ .  $\times 4,000$ .

variable, namely four groups of two bivalents (Fig. 58), one group of three with two groups of two bivalents (Fig. 57), and two groups of three bivalents (Fig. 56). In a few cases loose and doubtful associations of four bivalents have been found. The results are probably to be explained on the basis that the primary chromosome complement has been modified structurally compared with that of the swede, namely, that exchanges between non-homologous chromosomes have occurred with reduplication of one or both of the exchanged chromosomes. Secondary pairing could then occur between parts of chromosomes phylogenetically related. It should be weaker than that between whole chromosomes similarly related.

A possible structure of the chromosome complement may be arrived at if it be supposed that the original basic number is 6 as in the swede, and that the turnip is tetrasomic in respect of four of them, with the

proviso that two pairs of non-homologous chromosomes are segmentally interchanged. We may represent the two segments of each chromosome



FIGS. 51-8. Centenary green skin, yellow flesh turnip pollen mother-cells. Fig. 51, diakinesis with 10 bivalents;  $\times 3,300$ . Figs. 52-8, polar metaphase I plates showing various degrees of secondary pairing;  $\times 4,000$ . Fig. 52,  $8(1) + 1(2)$ ; fig. 53,  $6(1) + 2(2)$ ; fig. 54,  $7(1) + 1(3)$ ; fig. 55,  $4(1) + 3(2)$ ; fig. 56,  $4(1) + 2(3)$ ; fig. 57,  $3(1) + 2(2) + 1(3)$ ; fig. 58,  $2(1) + 4(2)$ .

by capital and small letters, in which case the original structure of the haploid was probably

Aa	Aa
Bb	Bb
Cc	Cc
Dd	Dd
Ee	
Ff	

while the altered structure may be

Aa	Ae
Bb	Bf
Cc	Cc
Dd	Dd
Ea	
Eb	

The maximum possible associations would be 2 groups of 3 bivalents and 2 of 2 bivalents. This has not been observed.

No cases of plates with univalents were seen in the many cells counted in the turnip; nor were any quadrivalents seen at diakinesis or other stages.

#### Swede-turnip Hybrid.

The material of this cross consisted of plants of swede  $\times$  (white  $\times$  yellow flesh turnip) and of yellow flesh swede  $\times$  Centenary yellow flesh turnip; the results from the two were identical in every respect. The results obtained in this hybrid are somewhat at variance with the conditions discovered by Morinaga (1929) (29) in crosses between *B. cernua*

( $n = 18$ ) and species having a haploid chromosome number of 10. He found regularly 10 bivalents and 8 univalents. The configurations in 17 cells that could be analysed completely, are summarized in Table VIII. It will be seen that 8 univalents and 10 bivalents represents the least amount of pairing observed. The number of bivalents varied from 7 to 12 in number, and in addition from 0 to 3 trivalents were seen in the cells fully analysed. The commonest configuration was 7 univalents, 9 bivalents and 1 trivalent (Fig. 60). Fig. 61 shows the greatest number of bivalents seen, viz. 12, together with 4 univalents; there is a persistent nucleolus lying beside the spindle. In Fig. 62 there are 8 bivalents, 2 trivalents, and 6 univalents; the profile view of metaphase I (Fig. 63) shows 3 trivalents, 7 bivalents, and 5 univalents. In very rare cases a quadrivalent was seen at diakinesis (Figs. 66 *a* and *b*); the two figured are respectively a branched chain and a ring of four.

TABLE VIII.

*Configurations of Chromosomes at Meiosis in Swede  $\times$  Turnip  $F_1$ .*

Types and numbers of associations.			Numbers of cells.
Univalents.	Bivalents.	Trivalents.	
8	10	—	2
7	9	1	6
6	8	2	3
5	7	3	4
6	11	—	1
4	12	—	1
			<hr/> 17

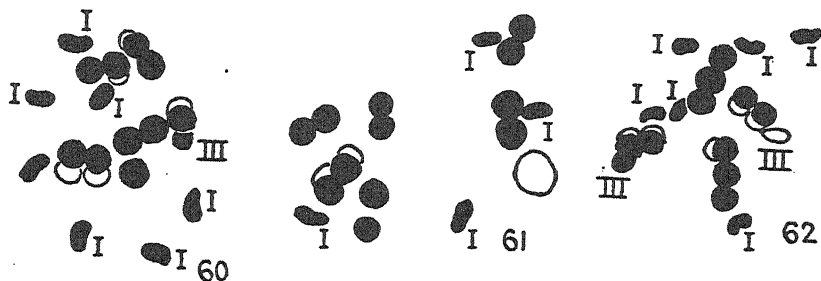
The relative frequencies of trivalent types is instructive; there were 6 Y-shaped (Figs. 63, 65 *b* and *c*), 16 chain (Figs. 63, 64, 65 *b* and *c*), and 2 ring and rod. The relatively greater number of the chain type may be taken to show a low chiasma frequency in the paired limbs or, more likely, structural complexity of the pairing chromosomes whereby they tend to pair with different chromosomes at their two ends. The chiasma frequency at metaphase I and diakinesis is somewhat lower than that in the turnip, which has 8 fewer chromosomes; the number of ring bivalents is also significantly reduced. The small size of the chromosomes, rendering observation difficult, precludes the extensive observations one would like on this point.

The frequencies of different degrees of secondary pairing of bivalents in the hybrid are summarized in Table IX, while a frequency polygon is shown in Diagram 1. Clearly the mode, five, is higher than in the turnip. This may be taken to indicate some autosyndesic pairing of swede chromosomes. Or, it may be adduced in support of structural differences between pairing swede and turnip chromosomes, whereby secondary pairing may occur when primary pairing fails. Probably the increase is a com-

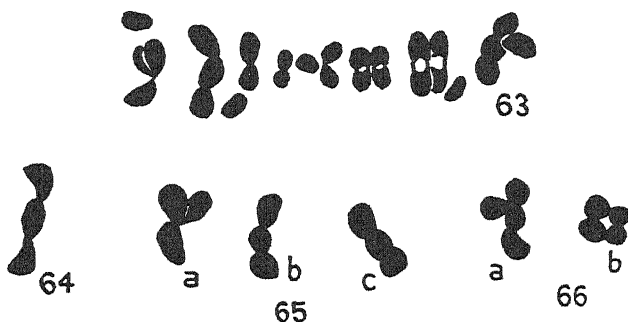
bination of the two causes. In support of structural complexity, it must be noted that univalents may be secondarily paired with bivalents or



FIG. 59. Centenary turnip, metaphase I in profile showing bivalent types and secondary pairing between morphologically similar bivalents.



FIGS. 60-2. Swede  $\times$  turnip  $F_1$  (ABK) pollen mother-cells at metaphase in polar view. Fig. 60, 1 trivalent, 7 univalents, and 9 bivalents showing secondary pairing to form  $1(1) + 3(2)$  with the remaining 2 bivalents associated with the trivalent; fig. 61, 12 bivalents and 4 univalents, the bivalents secondarily paired into  $2(1) + 5(2)$ , and 2 of the univalents secondarily paired with bivalents; fig. 62, 2 trivalents, 6 univalents, and 10 bivalents with secondary pairing of two sets of three bivalents and of each of the trivalents with a bivalent.  $\times 4,000$ .



FIGS. 63-6. Swede  $\times$  turnip  $F_1$  (ABK) pollen mother-cells at meiosis. FIG. 63, complete profile metaphase I showing 3 trivalents, 5 univalents, and 10 bivalents with some degree of secondary pairing; fig. 64, chain trivalent from profile metaphase I; fig. 65, trivalents from diakinesis nuclei respectively of Y(a) and chain (b, c) types; fig. 66, quadrivalents from diakinesis nuclei respectively of branched chain (a) and ring (b) types.  $\times 4,000$ .

sometimes with other univalents (cf. Figs. 61 and 62); the extent of this is difficult to gauge.

TABLE IX.

*Frequencies of Different Degrees of Secondary Pairing at Metaphase I in Swede  $\times$  Turnip  $F_1$ . N.B.—Secondary Associations of Univalents with other Structures not counted.*

No. of secondary associations per cell	3	4	5	6
No. of cells	1	4	5	6

The behaviour of the univalents is complex, and difficult to follow. Apparently they may separate at the first anaphase (Figs. 67-9) into their two constituent chromatids (note single chromatids in interkinesis nuclei in

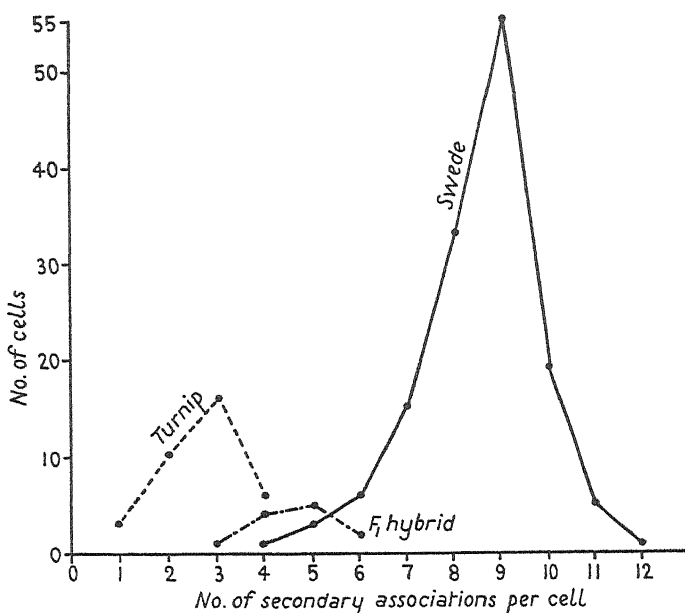
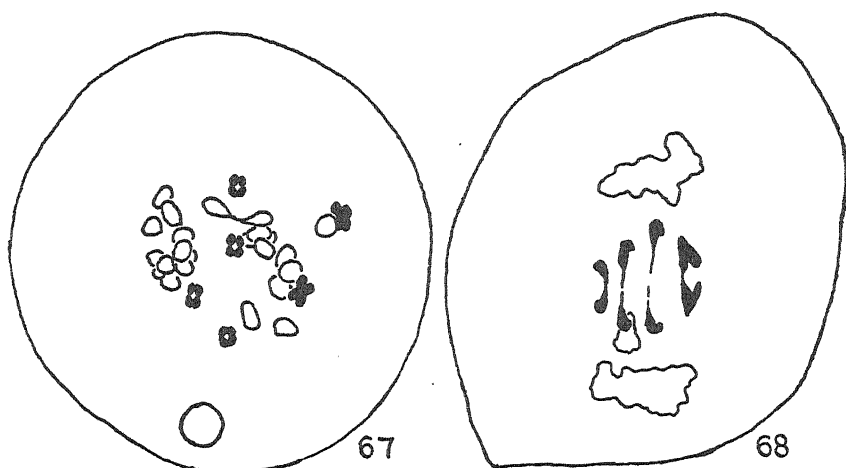


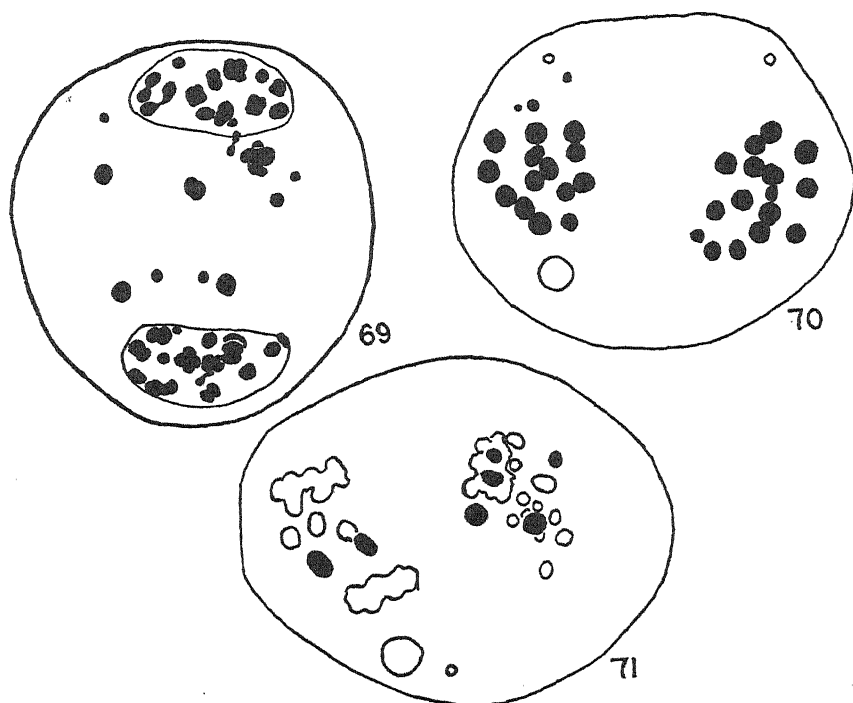
DIAGRAM 1. Curves showing the absolute frequencies of different degrees of secondary pairing at metaphase I found in samples of pollen mother-cells of swedes, turnips, and swede x turnip  $F_1$ .

Fig. 69), in which case they apparently pass at random to the poles at the second anaphase or are left out of the main daughter nuclei constituted at the end of meiosis. Such single chromatids regularly lag on the spindle at the second division (Fig. 71) or may divide a second time, especially if they are left out of the interkinesis nucleus. This second division of univalents may precede metaphase II (cf. Fig. 69) and the division of the univalent occur in any direction relative to the long axis of the original spindle; cases may be seen in Figs. 69 and 70. Double division of univalents, in this manner, provided their products are included in the major nuclei, may lead to gametes with chromosomes in excess of the normally expected numbers. There is no reason to suppose that gametes with less than 14 chromosomes will necessarily be non-functional. The hybrids showed a proportion (about 20 per cent.) of small, empty-looking pollen grains,<sup>1</sup> but the remainder appeared good though slightly variable in absolute size. Again, it is often clear from the constitution of the second metaphase plates that the univalents have mostly passed at random to one or other telophase group formed following the first anaphase; in the second

<sup>1</sup> Dr. V. McM. Davey informs me that this is a higher proportion of empty grains than he found in counts of several  $F_1$ 's.



FIGS. 67-8. Swede  $\times$  turnip  $F_1$  (ABK) pollen mother-cells at late anaphase I. Fig. 67, showing the daughter halves of lagging univalents beginning to disjoin; note nucleolus in cytoplasm; fig. 68, 1 lagging bivalent and 3 lagging univalents separating.  $\times 4,000$ .



FIGS. 69-71. Swede  $\times$  turnip  $F_1$  (ABK) pollen mother-cells. Fig. 69, telophase I with 2 nuclei and a number of laggard univalents, some divided and some undivided, left out of the nuclei; fig. 70, metaphase II with 14 and 16 chromosomes in the respective plates and a few fragments that have divided in the cytoplasm; note persistent nucleolus; fig. 71, late anaphase II with persistent nucleolus showing laggard univalents dividing.  $\times 4,000$ .

division such univalents separate, as easily as the rest of the chromosomes, into the two chromatids of which they are made up.

In addition univalents may lag on the first division spindle as well as on the second division spindle; in this case two possibilities arise. If

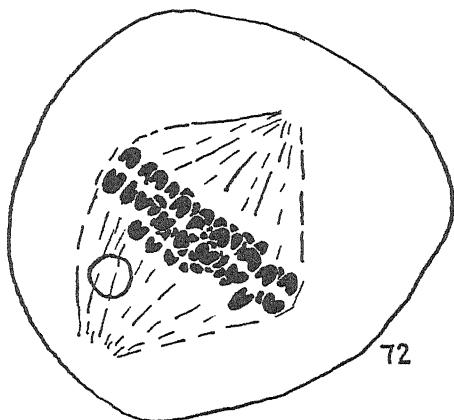


FIG. 72. Swede  $\times$  turnip  $F_1$  (ABK) pollen mother-cell at metaphase II showing a single plate in side view having about 28 chromosomes; this would follow a division I restitution nucleus.

there is a sufficiently continuous series of them from one telophase group to the other a restitution nucleus may be formed embracing the whole of the chromosomes. Formation of restitution nuclei in this way at either the first or second or both divisions in different cases is the explanation of the diads and triads noted by Morinaga (1929) in *B. cernua* ( $n = 18$ )  $\times$  *chinensis* ( $n = 10$ ) (29) and *B. cernua*  $\times$  *B. napella* ( $n = 19$ ) (30); in fact, he describes stages in the formation of restitution nuclei in the former hybrid. When restitution nuclei formed at division I divide, the gametes formed are diploid or approximately so; any deviation from diploidy must be traced to random distribution of chromatids formed from univalents that divided at division I. A metaphase II plate, with 28 dividing chromosomes, is shown at Fig. 72; it is the result of a division I restitution nucleus.

In other cases the two first telophase groups reconstitute independent interkinesis nuclei and the lagging univalents are left stranded between the two nuclei; usually each univalent then forms a separate small spindle on its own, and the two chromatids constituting it are separated to opposite poles (Fig. 69). Chromatids with such a history are still traceable during the second division well out in the cytoplasm away from the remainder of the chromosomes (Figs. 70 and 71).

Second metaphase plates with 13, 13 + half-chromosome, 13 + three half-chromosomes, 14, 15, and 16 chromosomes have been seen. The commonest arrangements appear to be 14 + 14, 13 + 15, and 13 + 14



with one chromosome left out of the two main plates. Accurate analysis is complicated by the difficulty of assessing the value of any particular body that is observed.

Diads and triads are relatively common amongst the tetrad groups of young pollen grains; the former (diads) represent about 5-8 per cent. of the whole.

TABLE X.

*Chromosome Numbers in Swede  $\times$  Turnip  $F_2$ .*

Number of chromosomes.																								
22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37-39	40	41	42	43	44	45			
Number of plants.																								
1	0	1	1	1	9	5	6	0	7	5	3	2	3	2	0	1	0	3	0	2	1			

The hybrid is highly self-sterile, but will give a few seeds. The chromosome numbers summarized in Table X were determined in 53  $F_2$  plants. Four modal points (cf. Diagram 2) may be distinguished, viz. (1) at 27-9, that is in the neighbourhood of the number 28 in the  $F_1$  hybrid parent; (2) at 31, an inexplicable result; (3) at 34-5, that is about 6 (the true basic number of the swede) more than the number in the parent (N.B. the excess number also corresponds to the average number of univalents at meiosis in the parent) which perhaps means that these zygotes are better balanced than certain others; (4) at 42 (relatively triploid) which is half as many again as the parent, and means that diploid (unreduced) gametes derived from restitution nuclei have been functioning. It is noteworthy that 7 out of 53 plants (about 13 per cent.) were approximately 'triploid', indicating a corresponding frequency of activity on the part of the unreduced gametes. Diads, however, account for only 5-8 per cent. of the 'tetrads', so that unreduced gametes are about twice as viable, or active, as are the sub-normal reduced ones. Mating of two unreduced gametes would give an amphidiploid, probably with a certain degree of variable pairing. Frandsen and Winge (1931) (7) have obtained amphidiploid plants from a somatically doubled inflorescence of an  $F_1$  plant; they seem to be quite fertile. Such 'tetraploids' should occur with a frequency of about 1.7 per cent. in a selfed (or better, outbred, since self-incompatibility factors are involved) population, if there are random chances for mating of haploid and diploid gametes.

Some  $F_2$  pollen mother-cell material, fixed by Dr. Davey, unfortunately yielded no stages. According to Nelson (1927) (32) the  $F_1$  swede  $\times$  turnip hybrid is fully fertile in reciprocal backcrosses with swede, and fairly so with turnip, while the  $F_1$  turnip  $\times$  swede is fairly fertile with swede but shows a low fertility with turnip. Chromosome numbers in the backcross

(swede  $\times$  turnip)  $\times$  swede are given in Table XI and graphically in Diagram 2. The figures indicate a peak development, by the  $F_1$ , of viable gametes with 14 chromosomes.

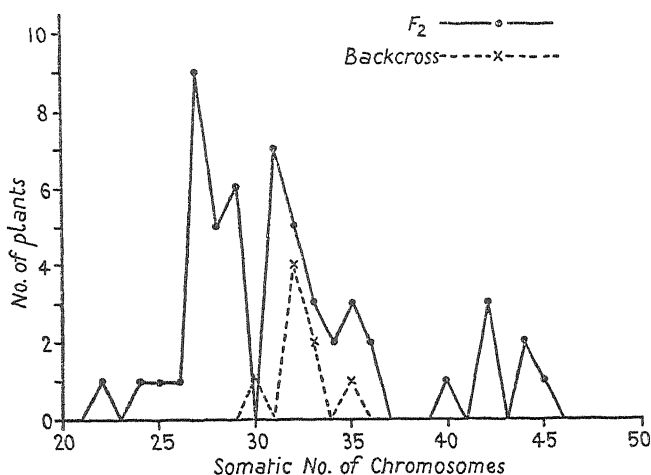


DIAGRAM 2. Curves showing the frequencies of different chromosome numbers in populations (a) of swede  $\times$  turnip  $F_2$ , (b) of (swede  $\times$  turnip)  $\times$  swede backcross.

TABLE XI.

*Chromosome Numbers in (Swede  $\times$  Turnip)  $\times$  Swede Backcross.*

No. of chromosomes	30	31	32	33	34	35
No. of plants	1	0	4	2	0	1

One plant of Dr. Davey's  $F_2$  swede  $\times$  turnip was markedly more fertile than the rest, and it was thought possible that it was an amphidiploid. Studies were made of root-tips from a number of seeds, and numbers fluctuating about 42, the 'triploid' number, were found. Seemingly, the 'triploid' individual has greater fertility than the diploid hybrid.

#### DISCUSSION.

It is possible, from the cytological facts described above, to reach certain conclusions with regard to the major problems defined earlier.

In the first place all the forms belonging to the swede alliance from a cytological point of view are closely related. They all have the same chromosome number and there are no detectable differences in the morphology of the different complements. At meiosis, pairing is perfect in the hybrids and there is no sign of the reduction in chiasma frequency that would be expected if there were qualitative or quantitative differences between the different complements.

Swede is a biennial crop but liable, like others, to 'bolt' or 'run to

seed' in its first year. This is largely conditioned by environmental factors, though, as in the sugar beet (9), hereditary factors are also involved. In addition, there is a second bolting type, the bulbless bolter, which sometimes appears as a rogue in crops and which breeds true to its bolting tendency. When the bulbless bolters appear rather abundantly in a crop, the value of it may be considerably diminished; this has sometimes led to legal actions, the seed merchant being sued for negligence. The defence has been that the bolter is a 'reversion'. This explanation is most unlikely, since white flesh bolters appear in yellow flesh swede crops, white flesh being dominant to yellow flesh. Actually, pure-bred swedes do not throw bulbless bolters at all. Lafferty (18) and Davey (2) have shown that these bolters consist of admixed swede-like rapes and hybrids of swedes and swede-like rapes. Some of the latter when selfed are found to segregate for flesh and skin colour. The cytological facts are entirely in agreement with the views of these authors.

Some turnip varieties, e.g. 'Bruce' and 'Fosterton hybrid', have been supposed to have swede ancestry. But the chromosome numbers of turnips and swedes, taken with the general facts of hybrid cytology, renders such an hypothesis extremely improbable. The two varieties named have similar somatic chromosome complements to that of admitted turnips, and must be regarded as merely mutant types.

The major problems of the duplicate (multiple) factors, the different basic numbers in different species, and the possibility of combination of swede and turnip to form a new hybrid species are really interrelated subjects. Secondary pairing, as it has been interpreted, involves the conception that different pairs of chromosomes in the same plant will bear factors (or more likely blocks of factors) in common. The occurrence of duplicate factors in *Brassica* is well established; the cytological basis is established now in the form of secondarily balanced polyploidy, that is polyploidy affecting different chromosomes unequally.

In *B. oleracea* ( $n = 9$ ), Pease concluded (34) that two duplicate factors,  $N_1$  and  $N_2$ , govern the non-hearting of kale, kohlrabi, and other non-hearting forms; Malinowski (23) believes that three duplicate factors are involved. The curly foliage of kale, as against the non-curling of the cabbage, was found by Pease (34) to be controlled by two factors,  $K_1$  and  $K_2$ . These factors have also been found in broccoli (Kristofferson (16)) and in kohlrabi (Malinowski (24)). Kohlrabi has duplicate factors governing 'bulbing'. Purple pigment in kohlrabi is controlled by two factors, there being a 9:7 ratio of purples to greens in the  $F_2$  following a cross with green savoy. Other doubtful cases are recorded, but the majority of the other known factors are represented but once in the genetical make-up of the germinal material. All these duplicate factors occur in an alleged diploid.

The incompatibility interrelationships in the cabbage, determined by

Detjen (5) are such as are shown by polyploids, a question that has been fully discussed by Lawrence (20). The latter, dealing with Kakizaki's results (13), has suggested that *B. oleracea* is partially polyploid and finds some support in suggestions of secondary pairing in Karpechenko's illustrations of meiotic metaphases in this species.

The genetical conditions in *B. oleracea* obviously point to a polyploid condition. The factors (or their allelomorphic ancestors) for non-hearting, for curly foliage, for pigmentation, and perhaps others, were present before the haploid number of 9 was established in these *Brassica* forms and were duplicated in the course of the establishment of  $n = 9$ . In this connexion, it is noteworthy that there is probable linkage (Pease (34)) between  $N_1$  and  $K_1$  and between  $N_2$  and  $K_2$ ; Malinowski's (23) findings of a negative correlation between degree of hearting and degree of curliness in the  $F_2$  of cabbage  $\times$  curly kale corroborate the suggestion. Secondary associations would then be expected to occur between those bivalents whose chromosomes bear the factors for non-hearting and curly foliage (and probably also one or more other pairs of bivalents). But such pairing between the bivalents would leave the random segregation of the bivalents concerned quite unaffected, so that dihybrid and not autotetraploid ratios would be expected.

The swede may be supposed to be a numerical allotetraploid derivative of *oleracea* forms, derived perhaps from crossing of two  $n = 9$  species with subsequent amphidiploidy. In that case it is feasible that the haploid chromosome set of *B. oleracea* is of the type:

A A  
B B  
C C  
D  
E  
F

Various basic numbers have been reported for *Brassica* in different species, viz. 8, 9, 10, 17, 18, and 19 (cf. Morinaga (27-31) and others). Probably all these are secondarily balanced numbers, and perhaps they represent different balances of the same primary number. The real basic (primary) number is 6 in the case of the swede ( $n = 18$ ). The turnip is probably based on the same primary number, but the types of the secondary associations found suggest a superimposed structural rearrangement of the parts. This is corroborated to some extent by the discovery of a higher degree of secondary pairing in the swede  $\times$  turnip  $F_1$  than in the turnip. A considerable amount of information could be obtained from a systematic examination of a number of different species—though the method would undoubtedly be complicated by any structural changes that have occurred. Some of the variant numbers found in *Brassica* may be due to fission or fusion of chromosomes, the occurrence of which might be established from studies

of somatic chromosomes; again the inquiry may be limited too much by the small size of the chromosomes.

The cytology of interspecific hybrids aids, too, in resolving relationships. Morinaga's studies (27-31) lead to preliminary conclusions. His results may be summarized briefly as follows:

Haploid nos. of parents.	No. of bivalents.	No. of univalents.
10 × 10	10	—
10 × 17	1-9	25-9
10 × 18	10	8
10 × 19	10	9
18 × 19	10	17

It is at once apparent that all the species have one particular group of ten chromosomes in common (assuming allosyndesis), and that the remaining eight chromosomes of the eighteen chromosome species (e.g. *B. cernua*, *B. juncea*) are different from the remaining nine chromosomes of the nineteen chromosome species (*B. Napella*). The hybrids with *B. oleracea* ( $n = 9$ ) would be especially instructive from this point of view. The evidence of the hybrids must be taken as proof of differences between the various members of the chromosome complements present in the various species, without, however, specifying whether the differences are qualitative or quantitative.

In a cross between species, with haploid numbers of 18 and 10, in which 10 bivalents and 8 univalents are commonly found, it is usually concluded that 10 chromosomes of the lower numbered species have paired with 10 chromosomes of the higher numbered species. This assumption is extremely incautious and depends upon the unproved premise that the chromosomes from the two species are incapable of autosyndesis. Further, the variable degree of pairing and the presence of trivalents in the swede × turnip  $F_1$ , taken with the novel finding that secondary pairing is more intense in the hybrid than in the turnip, suggests that the situation is very much more complex.

It is possible in cases where secondary pairing is traceable that there might be some formation of bivalents in a haploid plant. The nearest approximation to this is in Karpechenko's *Brassica* × *Raphanus*  $F_1$  (15) in which the 18 chromosomes, 9 derived from each parent, usually failed to pair and formed univalents only. Exceptionally a single bivalent was observed, but it is impossible to decide whether the pairing is allo- or autosyndetic. Secondary association is present in *B. oleracea* in Karpechenko's figures of polar metaphase, as pointed out by Lawrence (20). Winge's (36) drawing also points to the same conclusion. Hence, although there may be a strong enough generalized attraction between the phylogenetically related chromosomes, yet the particulate attraction is too discontinuous to allow at all frequent pairing. There must, however, be

some pairing, as is shown by the occasional presence of trivalent and quadrivalent associations in members of the swede group.

The same phenomenon is illustrated by the interspecific hybrids. It has been shown that there are as many as 8 and as few as 4 univalents; there were from 0 to 3 trivalents and from 7 to 12 bivalents. Clearly the frequency of univalents indicates a strong dissimilarity between the chromosomes; in all probability the chromosomes are homologous only over very short lengths, too short to give more than very occasional chiasmata. Further, pairing in one short region would interfere mechanically with pairing of other adjacent short sections. The usual statement of 10 bivalents and 8 univalents is probably more of an ideal than one of fact.

Secondary association therefore may be shown between chromosomes that have so little particulate attraction for one another over any considerable length of the chromosomes that chiasma formation between them is a rare event. Considerable reduplication in an acknowledged diploid clearly demonstrated in its haploid (Catcheside (1)) does not appear to interfere with normal pairing to any extent. For short reduplicated segments result in the formation of various bivalents and other associations in about 20 per cent. of the pollen mother-cells in the haploid, while corresponding multivalents are very scarce in the diploid. In this way, two chromosome pairs of different homologies may carry short blocks of identical genes, but the absence of extensive particulate affinity, prohibiting chiasma formation, makes an independent distribution practically compulsory. Only occasionally does this allopolyploid pairing become autopolyploid by reason of an unusual association of the chromosomes concerned.

Both the swede (*B. Napus*) and the turnip (*B. rapa*) have been shown cytologically to be secondarily balanced polyploids, the turnip probably being structurally more complex than the swede. It would be interesting to examine the occurrence and distribution of known factors between them. The swede shows dihybrid ratios for white flesh and bright lemon flowers (yellow flesh and dull buff flowers are recessive) and for purple neck (bronze or green top are recessive) (Kajanus (12); Davey (3)). Hallqvist (10) found a dihybrid factorial basis for leaf shape, the normal lobed leaf being dominant and entire leaf recessive; Davey (*in litt.*) has confirmed this. Sylven (35) has demonstrated four tones of yellow for the flowers of swede-like rape dependent on combinations of two pairs of factors. Bright lemon yellow is the dihybrid dominant, while a pale yellow colour appears in the presence of one factor. The corresponding factors in the turnip are monohybrid in the cases of flesh and flower colour and of skin colour. Kajanus (12) found also that the hairiness of swede leaves and the smoothness of turnip leaves were allelomorphs. Finally there are the two multiple factors  $L_1$  and  $L_2$  postulated by Kajanus (12) to be responsible for the

length of turnip roots. Though not extensive, the facts show that the swede and turnip have certain factors in common and that certain of them represented once in the turnip are duplicated in the swede. There are also genetical indications that the turnip is an allopolyploid.

We may now compare the primary and secondary chromosome numbers of the genus *Brassica* with the numbers found in other Cruciferae. The data presented by Manton (25), in addition to other scattered records, form a sufficiently sound basis for this. The primitive haploid number in the Cruciferae appears to me to be 7; but Manton concludes that the data are quite inconclusive. In a number of cases, however, she has observed that the fundamental chromosome number in a genus has been diminished, the clearest cases being in *Hesperis* ( $2n = 28, 26$ , and  $24$  in different species) and in *Matthiola* ( $2n = 14$  and  $12$  in different species). Related genera also have been found to show the same relationship. This change she interprets as aneuploid loss; but having regard to the characteristic inviability of organisms deficient in chromosomes or parts of chromosomes, this is exceedingly unlikely. A fusion of two non-homologous chromosomes, as in so many plants and animals, is more probable, and in fact seems to be distinctly supported by the existing data. In *Matthiola incana*, for instance, the 7 haploid chromosomes are roughly equal in length, whereas in *M. odoratissima* (cf. Manton (25), Fig. 35) one of the 6 haploid chromosomes is about twice the length of the remainder. Definitive evidence of fusion has been obtained in *Cardamine* by Lawrence (22) who finds 30, 14, and 16 somatic chromosomes in different species. The origin of a 6 from a 7 chromosome type by fusion in the phylogeny of the species of *Brassica* considered is a simple assumption; while the occurrence of a wide and more or less continuous range of haploid numbers in the Brassiceae clearly indicates crossing and the simultaneous establishment of a variety of derived secondary polyploid types. In contrast to fusion, fragmentation may also occur and would increase the basic number without disturbing the balance. These changes (fusion, fission, and change in balance) are apparently those that Manton has designated aneuploid change (loss or gain) in chromosome number.

Polyploidy, in *Brassica*, can arise in several ways. Karpechenko (15) has amply demonstrated the production of diploid and tetraploid (or approximately so) gametes in *Raphanus*  $\times$  *Brassica*  $F_1$  by (a) formation of restitution nuclei after divisions I or II giving diploid gametes, or after both together giving tetraploid gametes; (b) division of archesporial nucleus without division of cell, the spindles from the two nuclei amalgamating at metaphase I (syndiploidy). He also suggests (c) the possibility of somatic doubling as the result of incomplete mitosis in the archesporium with the formation of a giant nucleus, but records no cases, unless the embryo-sac mother-cell he mentions follows such a doubling of the chromosome number.

Fukushima (8) has traced this phenomenon in *B. japonica* 'Mizuna', finding groups of tetraploid and octoploid cells formed as the result of doubling in pre-archesporial cells, several cell generations before the reduction division. Its frequency here must be taken to indicate a presumably heritable racial character, perhaps a genic-environmental reaction. The tetraploid pollen mother-cell figured from the white flesh swede is probably the result of syndiploidy, two nuclei having amalgamated at prometaphase. For it shows (a) absence of univalents, trivalents, and quadrivalents, to be expected in what would be an autotetraploid nucleus had nuclear union been prior to chromosome pairing; (b) occurrence of 36 bivalents, such as would be expected if pairing took place in each of two diploid nuclei. Union at prometaphase on dissolution of the nuclear membranes would allow secondary pairing between bivalents derived from different nuclei. It is of importance to notice that the number of secondary associations in the tetraploid plate is very considerably more than twice the average in normal metaphase plates. This last is due undoubtedly to the close grouping consequent upon a relatively smaller equatorial spindle area at prometaphase resulting in a greater number of attractable bivalents coming within any particular bivalent's sphere of influence.

It should be noted that formation of exactly diploid balanced gametes is not a necessary corollary of the different methods of doubling except in the case of syndiploidy. The pairing capabilities of the chromosomes present before the doubling must be taken into consideration. For instance, if a univalent should separate into its constituent chromatids at anaphase I and then a restitution nucleus be formed, the single chromatids will then separate at random at anaphase II. Again, in the case of a plant possessed of homologous pairs of chromosomes, formation through somatic doubling of nuclei with double the number of chromosomes would at meiosis permit trivalent and quadrivalent association and so upset the regular formation of gametes and lead to some sterility.

Viable diploid gametes are, however, produced with no great infrequency in *Brassica*. This is clear when the chromosome numbers in the swede  $\times$  turnip  $F_2$  are considered. A number of the progeny are clearly relatively triploid (cf. Table X); one may therefore expect occasional tetraploid or nearly tetraploid plants. An amphidiploid has been obtained by Frandsen and Winge (7), who find it has 56 chromosomes and that it is fertile and true breeding. It originated in the  $F_1$  through somatic doubling, giving a fertile tetraploid branch. Its meiotic conditions would be interesting for, judging from the  $F_1$  hybrid, the amphidiploid should be at least partially autopolyploid. But competition between interchromosomal attractions may lead, as in *Primula Kewensis* (Newton and Pellew (33)), to auto- rather than allosyndesis, tending to eliminate quadrivalents in favour of bivalents only and so stabilizing the strain. In any case a regular proportion



of chromosomal aberrants may be expected; the economic usefulness of the strain will be in inverse ratio to this proportion, without reference to any other characteristics.

#### SUMMARY AND CONCLUSIONS.

The chromosomes of members of the swede and turnip groups of *Brassica* have been examined, and lead to the following conclusions regarding the problems considered.

1. The agricultural swede rogue known as the 'bulbless bolter' has a chromosomal constitution similar to that of swede and swede-like rape; it is usually a plant of the latter form, though sometimes a hybrid of the two. It agrees with the swede and swede-like rape in chromosome number (36), morphology of complement, chiasma frequency at meiosis, and degree and type of secondary association of bivalents. The hybrids of the three forms also agree with the parents in their cytological constants.

2. Certain turnips ('Bruce' and 'Fosterton hybrid') alleged to have swede ancestry are found to agree with other turnips in chromosome number (20) and in the morphology of their chromosome complements.

3. Swedes (and their relatives) usually form 18 bivalents at meiosis. The bivalents are uniformly spaced in the nucleus at diakinesis, but are secondarily paired on the equatorial plate at metaphase I. The minimum number of groups of bivalents is 6, which is therefore the primary basic chromosome number. Of these 6 groups found when secondary pairing is at a maximum, 3 are made up of 4 bivalents, and 3 of 2 bivalents each. The swede is therefore a secondarily balanced polyploid. The frequencies of different degrees of association lie on a unimodal polygon, the mode being at 9. Exceptionally, trivalents and quadrivalents have been seen; these support a polyploid interpretation.

4. Turnips form 10 bivalents at meiosis, and also show secondary pairing at metaphase I. They are therefore secondarily balanced polyploids, but probably with structural change of certain chromosomes superimposed.

5. Secondary balanced polyploidy is a sufficient explanation of the duplicate factors known in cabbage ( $n = 9$ ), turnip ( $n = 10$ ), and swede ( $n = 18$ ).

6. The  $F_1$  hybrid of swede and turnip shows a variable degree of pairing, with some formation of trivalents and rare quadrivalents. It is not possible to say whether all the turnip chromosomes normally pair with 10 swede chromosomes. The occurrence of various multivalents, the polyploid nature of the parents, and the higher degree of secondary pairing in the hybrid than in the turnip, suggest that the usual simple allosyndetic interpretation is open to grave suspicion.

7. Aberrations of meiosis in the hybrid lead to viable gametes with a variety of chromosome numbers; 'diploid' gametes with 28 chromosomes arise following a division I restitution nucleus. The chromosome numbers of a series of  $F_2$  plants have been determined. The chance of stabilizing swede  $\times$  turnip as an amphidiploid must depend upon the competition in chromosome pairing. If autosyndetic pairing is the rule, the plant should breed true; but if allosyndetic pairing between swede and turnip chromosomes also occurs to any extent, unbalanced forms may be formed too frequently. The results of Frandsen and Winge (7) suggest that their *Brassica napocampestris* may be quite a stable species.

8. The phylogeny of the Cruciferae has involved cases of fusion, fission, and other structural changes besides polyploidy and change in balance as in *Brassica*.

I must record, in conclusion, my indebtedness to Professor J. M. F. Drummond who rendered very considerable help in the earlier stages, to Professor R. R. Gates, who has made many useful suggestions, and especially to Dr. V. McM. Davey who has given me a great deal of help with innumerable matters of agricultural and genetical significance, and to whom I am indebted for practically all the material examined.

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# On the Presence of Cellulose and its Distribution in the Cell-walls of Brown and Red Algae.

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With five Figures in the Text.

THE cell-walls of some marine algae contain substances which do not occur in other groups, such as the fucoidin and algin of the Phaeophyceae and the mucilage of the Rhodophyceae. These facts, together with the circumstance that a study of the literature revealed some uncertainty as to whether true cellulose occurs at all, suggested the desirability of a critical macro- and microchemical inquiry into the distribution of cellulose in these plants. Czapek (2) states that cellulose occurs in the walls of *Fucus*. In 1915 Kylin (4) stated that cellulose was present in the walls of *Laminaria digitata*, *L. saccharina*, *Ascophyllum nodosum*, and *Fucus vesiculosus*, as he obtained a blue colour on treatment with iodine and sulphuric acid. According to Czapek, however, this colour is also given by a carbohydrate known as 'fucin', a substance allied to alginic acid. Kylin proceeded to estimate the substance he held to be cellulose by heating with 1.25 per cent. sulphuric acid, followed by 1.25 per cent. caustic soda, but he does not appear to have carried out any tests on the product of this treatment to establish its cellulose nature.

In 1931, Ricard (6) treated *Laminaria flexicaulis* and *L. saccharina* in the same way, but was unable to obtain a blue coloration with iodine and sulphuric acid.

As regards the red algae, Czapek states that the tissue of *Sphaerococcus crispus* has been shown to consist of thick cellulose walls with some other intercellular substance, but he adds that the amount of cellulose in red algae would not appear to be large.

So far as can be ascertained, the evidence for the presence of cellulose in the walls of brown and red algae rests on no firmer basis than the test with iodine and sulphuric acid in the original weed, which is also positive with fucin, and sometimes on the colour given with chlor-zinc iodide, as, for example, in Walter's (9) paper in 1923.

In 1922, one of the present authors (B.R-W) isolated cellulose from the red alga *Chondrus crispus* and showed it to be soluble in cuprammonia (7).

#### MACROCHEMICAL EXAMINATION.

The following algae were investigated: Among the browns, *Laminaria saccharina*, *L. digitata*, *Fucus serratus*, *F. vesiculosus*, *Ascophyllum nodosum*, *Pelvetia canaliculata*, *P. canaliculata* forma *libera*; among the reds, *Corallina officinalis*, *Bostrychia scorpioides*, *Chondrus crispus*, and *Rhodomenia palmata*.

The brown seaweeds were selected with regard to their vertical distribution. *Pelvetia canaliculata* forma *libera* is exposed to the air during most of its life, being immersed only during high spring tides, *P. canaliculata* is immersed for a short time every day, *Ascophyllum* and *Fucus vesiculosus* grow in fairly shallow water, *F. serratus* in water up to a depth of about 6 feet, and *Laminaria* spp. are only exposed during the lowest spring tides.

The red algae were chosen for their difference of habit; *Corallina* is an example of a calcareous alga; *Bostrychia*, which is usually found in the same type of habitat as *P. canaliculata* forma *libera*, presents a very much dissected, filiform type of thallus; *Chondrus crispus* has a fleshy branched thallus, and *Rhodomenia palmata* has a flat, thin, palmate thallus.

#### *Estimation of cellulose.*

For most plants the procedure was the same, the crude fibre being isolated in the usual way. The weed was first heated for half an hour with 1.25 per cent. sulphuric acid under a reflux condenser, then filtered off on a sinter funnel, to avoid possible contamination with cellulose from filter paper, washed free from acid with hot water, and then heated for half an hour with 1.25 per cent. caustic soda. The product was again filtered, washed free from alkali, dried to constant weight in a steam oven and the ash content determined, the weight of organic material being obtained by difference. This treatment was varied for *Corallina officinalis* owing to the incrustation of calcium carbonate, the weed being first soaked in 10 per cent. acetic acid and the product being estimated on the carbonate-free weed. In those brown algae which contain appreciable amounts of fat the weed was first freed from fat by ether extraction (3: 8).

#### *Qualitative tests.*

In addition to estimating the crude fibre, another sample was prepared

for qualitative examination, and the following tests were applied to establish the presence of cellulose: first the colour test with iodine and sulphuric acid; secondly, solubility in cuprammonia. The substance was dissolved in cuprammonia, and subsequently recovered by the addition of sulphuric acid. Thirdly, a sample of acetyl cellulose was prepared by the method described by Barnett (1): this was purified by solution in chloroform, filtered, and recovered by evaporation. If all these tests were positive the product was concluded to be cellulose. From all the red algae examined a clear white product was obtained which dissolved easily in cuprammonia and in the acetyl cellulose reagents. The same held for the two species of *Laminaria* examined, but considerable difficulty was experienced at the outset in obtaining from the fucoids a 'crude fibre' in a condition suitable for establishing the presence of cellulose. The product obtained was dark brown, and resisted the solvent action of cuprammonia and the acetylating reagents. It was subsequently found that this difficulty was due to the fact that the material employed had been collected for some time and kept in an air-dried condition, and it was only on repeating the experiments on a freshly collected sample, still wet with sea-water, that a pure, white 'crude fibre' was obtained, which yielded to the solvent action of the reagents above named.

With such fresh material, however, the procedure had to be slightly modified since the wet material could not be extracted with ether to remove fat. It was therefore boiled directly for one hour with 5 per cent. caustic soda, then for half an hour with 2.5 per cent. sulphuric acid. The product gave a good blue colour with iodine and sulphuric acid. In this connexion it may be noted that the iodine solution takes some time to diffuse into the cellulose and a better result is obtained by teasing out the material in the solution. This may account for Ricard's failure to get the blue colour with his products.

The two facts, the importance of using fresh material and the slowness of the diffusion of iodine, account for the discrepancies in our preliminary work and probably also for the divergent results obtained by other authors.

#### CONCLUSIONS.

The presence of cellulose was established macrochemically in the cell-walls of the following red and brown algae, since all three tests employed were positive: *Corallina officinalis*, *Bostrychia scorpioides*, *Chondrus crispus*, *Rhodymenia palmata*, *Laminaria saccharina*, *L. digitata*, *Fucus vesiculosus*, *F. serratus*, *Ascophyllum nodosum*, *Pelvetia canaliculata*, and *P. canaliculata* forma *libera*.

The results of the quantitative experiments were as follows:

From *Corallina* 15.2 per cent. of cellulose was obtained, *Bostrychia*

yielded 2.6 per cent., *Chondrus* yielded 2.2 per cent., *Rhodomenia* 2.1 per cent., *Laminaria saccharina* 5.7 per cent., and *L. digitata* 3.7 per cent.

#### MICROCHEMICAL EXAMINATION.

At the outset the staining methods described by Mehta (5) and others were tried on sections of the thallus of various fucoids, but soon discarded since the results were inconsistent and indicated physical rather than chemical constitution.

To remove the non-cellulose constituents of the cell-walls, sections were heated in a 2.5 per cent. solution of sodium carbonate in a boiling water bath for one hour, washed till free from alkali, again heated for an hour in a boiling water bath with 2.5 per cent. sulphuric acid, and finally washed till free from acid. Thus the non-cellulose constituents were removed, and the now very fragile sections were tested with iodine and sulphuric acid. Chlor-zinc iodide was not used, since experience has shown it to be an unreliable reagent.

#### *Phaeophyceae.*

*Fucus vesiculosus* (Fig. 1) may be taken as a type of the brown algae examined. Cellulose was found to occur in the cell-walls of all the three main zones of the thallus, and was distributed as follows: in the peripheral zone the cells of the outer layer have a marked cap-like thickening of cellulose in the exterior wall, but the rest of the wall is either devoid of cellulose or has only a thin lining layer. The cell-walls of the inner layers of the peripheral zone have a cellulose lining which increases in thickness with age. In the cortical zone the walls are fairly thick, but the amount of cellulose is comparatively small and is concentrated in thin concentric layers, giving a characteristic stratified appearance when tested with iodine and sulphuric acid. In the medullary zone the large primary cells are thick walled but with only a small amount of cellulose, concentrated in very thin concentric bands, generally two to four in number. The descending hyphae are narrow, with thick walls which give a marked cellulose reaction but little or no stratification. *F. serratus* agrees with *F. vesiculosus*.

In *Ascophyllum nodosum* (Fig. 2) the outer and inner walls of the superficial cells have marked cellulose caps, whilst the lateral walls are always devoid of cellulose. The large thick-walled medullary cells show a slight diffusion of cellulose throughout, with a marked concentration in numerous concentric bands.

*P. canaliculata* (Fig. 3) and its marsh form *libera* differ from *F. vesiculosus* in the absence of cellulose, not only from the lateral and basal walls of the superficial cells, but also from all the other cells of the peripheral



zone. The cells of the cortical and medullary regions are poor in cellulose, and the number of laminae, as far as our observations go, is generally less than, and rarely exceeds, three.

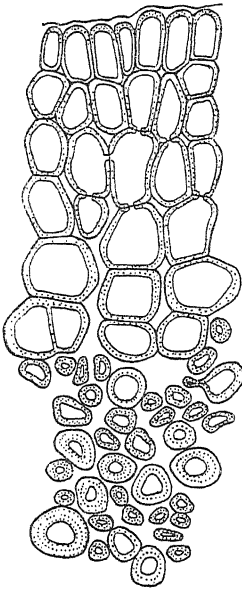


FIG. 1.

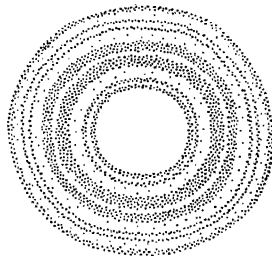


FIG. 2.

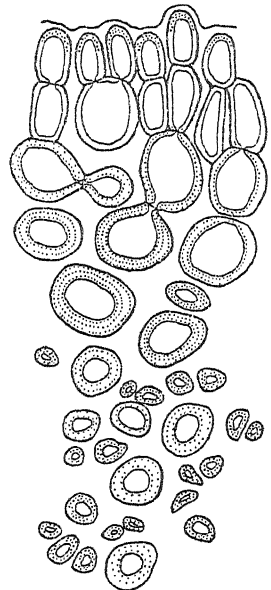


FIG. 3.

FIGS. 1-3. Fig. 1. *Fucus vesiculosus*. Transverse section of thallus. In this and the succeeding figures the dots represent cellulose. Fig. 2. *Ascophyllum nodosum*. Medullary cell showing stratification of the cellulose (transverse section). Fig. 3. *Pelvetia canaliculata*. Thallus (transverse section).

In *L. saccharina* and *L. digitata* the concentration of cellulose in the outer walls of the peripheral cells is less marked when compared with the former plants. The inner peripheral and cortical cells have more cellulose in the inner parts of the walls; all cells have a cellulose lining.

### *Rhodophyceae.*

*Chondrus crispus* (Fig. 4) belongs to the 'Fountain Type' of red algae. All cells of the peripheral zone have a very thin cellulose wall. Those of the central mass have well defined and often thick walls giving a very good cellulose reaction. No stratification of the cellulose occurs. In *Rhodymenia palmata* cellulose occurs in all the cell-walls. Before examining *Corallina* the calcareous incrustation was removed by soaking the specimens overnight in 10 per cent. acetic acid. Cellulose occurs in all the cell-walls. The 'link' cells differ from the other cells of the thallus in being impregnated with callus, as is indicated by the characteristic reaction with corallin soda.

The cell-walls of *Bostrychia* (Fig. 5) consist largely of cellulose which tends to be concentrated towards the inner parts of the cell, but with no stratification.

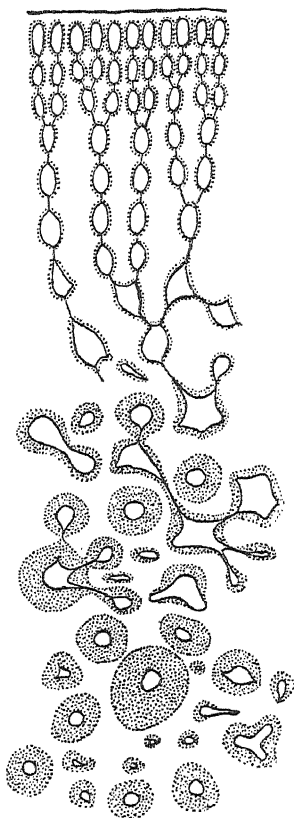


FIG. 4.

FIG. 4. *Chondrus crispus*. Thallus showing distribution of cellulose (transverse section).

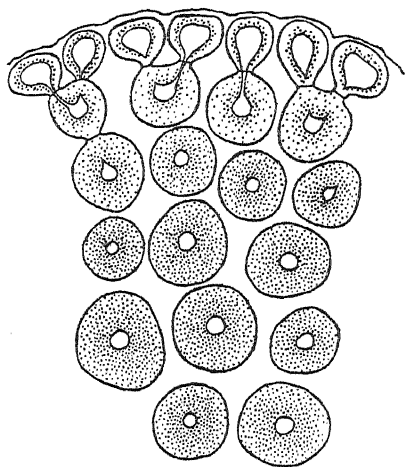


FIG. 5.

FIG. 5. *Bostrychia scorpioides*. Thallus showing distribution of cellulose (transverse section).

#### SUMMARY.

1. The presence of cellulose has been established macrochemically in the cell-walls of four red and seven brown algae.
2. The distribution of the cellulose in the tissues of the various algae examined has been worked out.

In conclusion, the authors wish to thank Professor T. G. Hill and Dr. P. Haas for their interest and advice during the progress of the work.

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# On Carpel Polymorphism. VI.

BY

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With one hundred and fifty-four Figures in the Text.

THE family Rutaceae which forms the chief subject-matter of the present account was selected for detailed investigation for the reason that it includes types characterized by features which, on the hitherto accepted interpretation of the floral ground-plan, appear as so many anomalies, or at best remain without explanation. In the account which follows it is shown that on the interpretation here adopted these several features fall into line and present no difficulty. Comparison of the Zygo-phylaceae, Meliaceae, Cneoraceae, and Erythroxylaceae, with the Rutaceae makes it evident that in these families also an interpretation on the same lines meets all the facts. In the course of treating the problem of ob-diplostemony a brief reference is also made to the unrelated family Stachy-uraceae, which affords an unusual and instructive case of diplostemony.

## Rutaceae (Figs. 1-88).

In the Rutaceae the flower is very generally ♂, with the full number of whorls and with a ground-plan isomerous throughout. Nevertheless even among types agreeing in these particulars, so many variations occur in certain anatomical characters, especially in the case of the androecium and gynoecium, as to make it necessary to study each genus separately in order to arrive at an understanding of the interrelations of the several floral whorls.

The material available for investigation included the following thirty-three species representing twenty-five genera :

<i>Adenandra uniflora</i> Willd.	<i>B. heterophylla</i> F. Muell.
<i>Aegle sepiaria</i> DC.	<i>B. megastigma</i> Nees.
<i>Agathosma imbricata</i> Willd.	<i>Calodendrum capensis</i> Thunb.
<i>Barosma crenulata</i> Hook.	<i>Choisya ternata</i> H.B. & K.
<i>Boronia fastigiata</i> Bartl.	<i>Citrus Aurantium</i> L.

<i>C. decumana</i> Murr.	<i>Ptelea trifoliata</i> L. ♂ and ♀.
<i>Cneoridium dumosum</i> Hook. f.	<i>Ruta bracteosa</i> DC.
<i>Coleonema album</i> Bartl. & Wendl. f.	<i>R. graveolens</i> L.
<i>Correa speciosa</i> Ait.	<i>Skimmia japonica</i> Thunb.
<i>Dictamnus Fraxinella</i> Pers.	<i>S. Laureola</i> Sieb. & Zucc.
<i>Diosma succulenta</i> Berg.	<i>Thamnosma montana</i> Torr. & Frém.
<i>Eriostemon intermedius</i> Hook.	<i>Toddalia aculeata</i> Pers.
<i>Evodia hupehensis</i> Dode.	<i>Triphasia trifoliata</i> DC.
<i>Feronia elephantum</i> Correa.	<i>Zanthoxylum Bungei</i> Planch. ♀.
<i>Phebalium argenteum</i> Sm.	<i>Z. fraxineum</i> Willd. ♂.
<i>Phellodendron japonicum</i> Maxim. ♂.	<i>Z. planispinum</i> Sieb. & Zucc. ♀.
<i>P. amurense</i> Rupr. ♀.	

The results of an examination of the whole floral anatomy of the above types, in so far as they concern the problems here dealt with, are given below. It will be convenient, however, before proceeding further to give some particulars respecting the accompanying figures. All are from transverse sections taken when in series from below upwards, except Figs. 85–8. All figures of sections of any one species are magnified to the same scale except Fig. 53, which is more highly magnified than Figs. 54, 55, and Figs. 119, 120, which are more highly magnified than Figs. 121–25. The same explanatory abbreviations being employed throughout, the complete list is given here in order to avoid repetition.

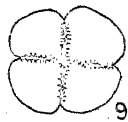
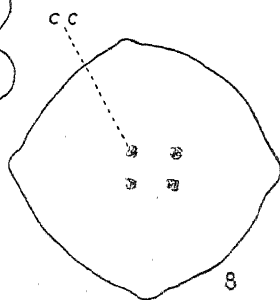
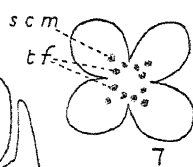
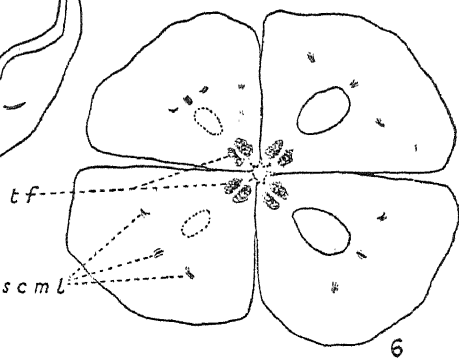
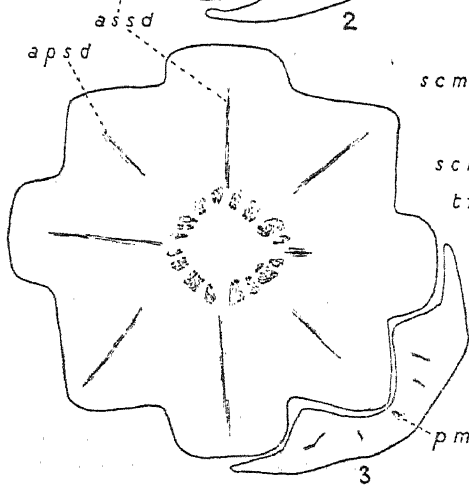
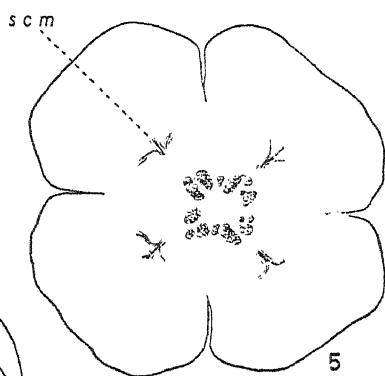
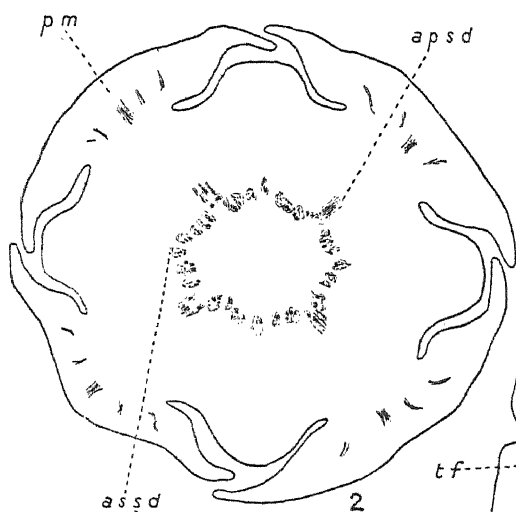
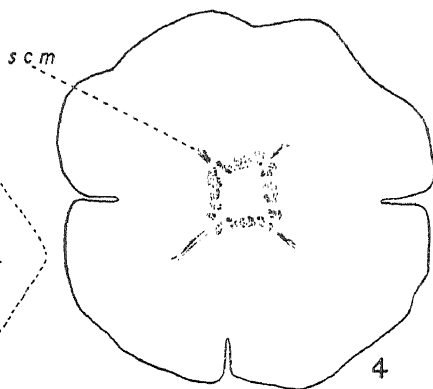
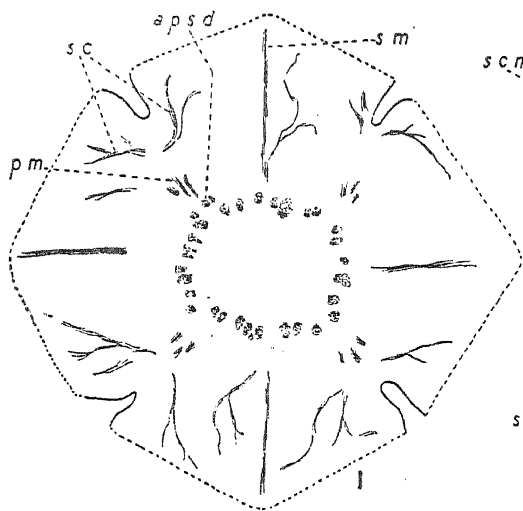
<i>a.p.d.</i> antepetalous disc strands	<i>o.c.</i> oil-containing cavity
<i>a.p.s.</i> antepetalous stamen bundles	<i>ov.b.</i> ovule bundle
<i>a.p.s.d.</i> antepetalous conjoined stamen-disc bundles	<i>p.</i> petal
<i>a.s.d.</i> antesepalous disc strands	<i>p.m.</i> petal midrib
<i>a.s.s.</i> antesepalous stamen bundles	<i>p.s.</i> petal-stamen cord
<i>a.s.s.d.</i> antesepalous conjoined stamen-disc bundles	<i>pl.b.</i> placental bundles
<i>c.</i> canal	<i>r.l.</i> radius of loculus
<i>cc.</i> carpel cord formed of the conjoined bundles of $\frac{1}{2}$ to $\frac{1}{3}$ carpels	<i>r.p.</i> radius of petal
<i>c.f.p.</i> cord formed of a false pair of bundles, the two members of the pair belonging to different carpels	<i>r.s.</i> radial split
<i>c.t.</i> conducting tissue	<i>s.</i> space
<i>d.</i> disc	<i>s.c.</i> sepal commissural marginal veins
<i>d.s.</i> disc strands	<i>s.c.b.</i> sterile carpel bundle
<i>f.c.b.</i> fertile carpel bundles	<i>s.c.m.</i> sterile carpel midrib
<i>f.c.c.</i> fertile carpel cord	<i>s.c.m.l.</i> sterile carpel midrib and laterals
<i>f.c.m.</i> fertile carpel midrib	<i>s.m.</i> sepal midrib
<i>g.</i> gynophore	<i>t.b.</i> twin bundles, each = one placental strand $\pm$ half the vascular complement of the neighbouring sterile carpel
<i>g.s.</i> 'gynobasic' style (filament or column)	<i>t.c.</i> trunk cord for sepal commissural marginals, petal midrib, and antepetalous stamen bundle
<i>m.v.c.</i> marginal veins of sterile carpels	<i>t.f.</i> twin fertile strands of one carpel
	<i>x.y.</i> limit of one unit stigma

*Calyx and corolla.* In some types the sepals receive only the midrib bundle, which may remain unbranched (*Agathosma*, Figs. 19–22) or may give rise to true lateral branches (*Cneoridium*, *Coleonema*, *Diosma*, *Ptelea*,

*Ruta*, *Skimmia*, *Triphasia*). In other types the vascular supply is more abundant. In these cases, in addition to the midrib bundle, the sepals receive commissural marginal veins. These veins arise in characteristic manner, trunk cords, which turn out on the petal radii, breaking up and giving rise to either a petal-stamen cord or a petal-midrib bundle and a calyx component which, on dissociation, immediately forks, the two resulting branches passing to the neighbouring sepal to right and left, respectively (*Adenandra*, *Barosma*, *Boronia*, Figs. 1, 11, *Calodendrum*, Figs. 24, 25, *Choisya*, *Correa*). This mode of origin of sepal marginal veins from commissural trunk cords is widespread, and has been described in earlier accounts of the Cruciferae, Caryophyllaceae, and Primulaceae, and of individual genera in other families (see 11, pp. 183, 184).

*Androecium*. The androecium is typically two-whorled and isomerous. In a few types, e.g. *Diosma*, *Skimmia*, *Toddalia*, *Phellodendron*, only the antesepalous members are developed. Both whorls may be fertile, or one may be fertile and the other sterile. In the latter case, full development is attained by the antesepalous whorl in some species, by the antepetalous in others, even sometimes within the same genus (*Boronia*). This difference in fertility may obtain in both diplostemenous and obdiplostemenous forms. All members of the androecium receive a vascular strand, whether fertile or sterile. Only in a minority of cases do the bundles for *both* staminal whorls leave the central cylinder independently of those for the perianth. Among such types we find some (*Calodendrum*, Fig. 25, *Ruta*, *Triphasia*) in which the antesepalous, and others (*Boronia*, Figs. 2, 10, 11) in which the antepetalous, bundles are the first to turn outwards. In the great majority of species the antesepalous stamen bundles pass out independently, while those for the antepetalous members emerge conjoined with the petal midribs, and so are carried out earlier. As a rule this reversal of the normal scheme of alternation in the out-turning of the stamen bundles is associated with obdiplostemony, but in Rutaceae sinuosities in the outline of the disc may lessen or altogether nullify the obdiplostemonous effect which would otherwise result. The extent to which disc shape may affect stamen position is seen at its fullest, perhaps, in Stachyuraceae (see later, p. 683 and Figs. 135, 136).

*Disc*. Beneath the gynoecium, surrounding it and fused with its lower portion, there is usually a conspicuous cushion of tissue—the disc, often furnished with numerous vascular strands, which, however, in many types are evidently undergoing degeneration. These strands may constitute definite antesepalous or antepetalous groups, or they may arise irregularly at numerous points on the circumference of the central cylinder. The strands may issue independently after the exit of the stamen bundles and before the emergence of those for the outer carpel whorl (*Adenandra*, *Barosma*, *Ruta*). Or they may be carried out conjoined with the bundles for each stamen





whorl, becoming dissociated as these bundles make their way to the periphery (*Boronia*, Fig. 12, *Citrus*, *Choisya*, *Dictamnus*, *Ptelea*, Fig. 51). In some cases where the disc remains fused with the gynoeceum above the level of origin of the sterile carpel bundles, the innermost of these strands may extend outwards for so short a distance that when at a slightly higher level disc and ovary become disjoined, a few are left on the inner (i.e. gynoeceal) side of the split and continue up in the wall of the ovary (Figs. 56-9).

*Gynoeceum.* The gynoeceum, which has hitherto been held to consist of a single whorl of valve carpels, exhibits extreme variety in form and in the degree of union and relative development of its component parts. The ovary may be sessile on, or sunk in, a well-developed disc, or it may be carried up on a long gynophore (*Calodendrum*, *Thamnosma*). In a few genera it is completely syncarpous, with sessile stigmas (*Toddalia*), or with a terminal style column springing from the summit of the ovary, being then either multilocular throughout (*Triphasia*) or becoming unilocular above (*Aegle*, *Citrus*, *Feronia*). In the great majority, however, the ovule-bearing portion becomes cleft radially to the centre into as many separate ovaries as there are loculi (spurious apocarpy). As the pith tissue disappears the individual ovaries thus formed only remain coherent through the union of the more or less 'gynobasic' style filaments into a single column. These individual ovaries have hitherto been considered to be monocarpellary. In isomerous types these supposedly single carpels are almost invariably superposed upon the petals, but in one genus among those investigated they were found to be antesepalous. The style filaments, when distinct,

FIGS. 1-9. *Boronia heterophylla* F. Muell. 1. Flower base after the emergence from the central cylinder of the orthogonal sepal midribs, and the dissociation of the diagonal trunk cords into the sepal marginals and branching petal midribs. [The distal portion of each sepal has been cut away.] 2. The same after the exertion of the sepals. At the periphery the four petals not yet exerted. In the centre the residual vascular ring from which the four antepetalous stamen-disc cords are about to emerge; the corresponding antesepalous cords are defined but have not yet turned outwards. 3. The same after exertion of the petals, one of which is shown in position. The cords which dissociate later into the antepetalous stamen bundles and disc strands have left the central cylinder, those for the antesepalous stamen bundles and disc strands which turn out a little later, are seen on the alternate radii. 4. The flower after exertion of the perianth and both stamen whorls. At the periphery disc tissue becoming 4 lobed through indentations in the orthogonal planes. In the centre the residual vascular cylinder from which the sterile carpel midribs are emerging on the radii of the petals. 5. The same after the sterile carpel midribs have left the central cylinder; each is giving rise to a pair of lateral veins. The lobing of the disc tissue seen in 4 has extended inwards along the mid-line of each fertile carpel. In the centre the residual vascular elements are becoming organized into the twin fertile strands of the inner carpels. 6. The gynoeceum surrounded by a thin ring of disc tissue at the level of origin of the loculi, showing extension of the radial splitting, and the demarcation of the inner boundary of the carpels from the pith preparatory to complete separation into four distinct ovaries (spurious apocarpy), each composed of one sterile carpel together with half a fertile carpel on each side. The twin bundles of each fertile member have diverged and now lie close to the adjacent bundle of the neighbouring carpel on each side, thus giving rise to false pairs. 7. The individual ovaries reunited at the level of transition to the single style column, each showing a sterile carpel midrib and the bundle proper to half the fertile carpel on each side. 8. Base of the large 4-sided columnar stigma. The three separate bundles seen in each ovary in 7 have fused into a single cord. 9. Apex of the same showing four defined lobes. (The lobes are centred over the loculi and hence are antepetalous, not antesepalous as incorrectly stated in some accounts. See also Fig. 85 B.)

whether springing from the summit of the ovary or 'gynobasic', are always superposed upon the loculi, and hence in isomerous types, with the one above-mentioned exception, stand in line with the petals. The same is generally true also respecting the position of the stigma lobes. But in the genus *Boronia*, while the lobes of some species (*fastigiata*, *heterophylla*) undoubtedly stand in this position (Figs. 85 A, 85 B), those of other species (*megastigma*, *Purdieana*) have the appearance of being superposed on the sepals (Fig. 85 C), and have been so described, though both groups have the same ground-plan. This appearance is found, however, to be delusive (see later, pp. 653-6.)

Despite these many variations in outward appearance and orientation, an examination of the vascular anatomy considered in conjunction with the outward form shows that in Rutaceae, as in all other families so far investigated, the gynoecium is invariably composed of *two* carpel whorls, the outer sterile, the inner fertile. The midrib bundles of the sterile carpels are rarely well developed and are not infrequently lacking; those of the fertile members are even more rarely traceable. Two circumstances appear likely to have contributed to this result. In the first place, the base of the ovary is commonly encircled by, and undelimited from, a thick mass of disc tissue. This tissue frequently contains a considerable number of vascular strands, though in many forms they are evidently in process of degeneration. They may, indeed, remain entirely undifferentiated; even where differentiation takes place the lowest portion connecting with the central cylinder often remains unligified. A slight further decrease in general vigour in the same region at a later stage might well lead to a similar result in regard to the outgoing carpel bundles. Secondly, a feeble development of the carpel midribs seems very generally to characterize types with abundant secretion, whether of oil or mucilage, and the ovary in the Rutaceae, like the other organs of the plant, is usually furnished with an abundance of secretory cavities. We meet with the same association in the Malvaceae, which includes genera rich in mucilage.

The sterile carpels of most genera appear to be of the consolidated type, but in some in which the midribs are well developed, at least at the base (*Boronia*, Figs. 5, 6, *Eriostemon*, *Phebalium*, *Ptelea*, Fig. 52, *Zanthoxylum*, Figs. 40-2, 44, 45), the mode of branching is that characteristic of the valve type.

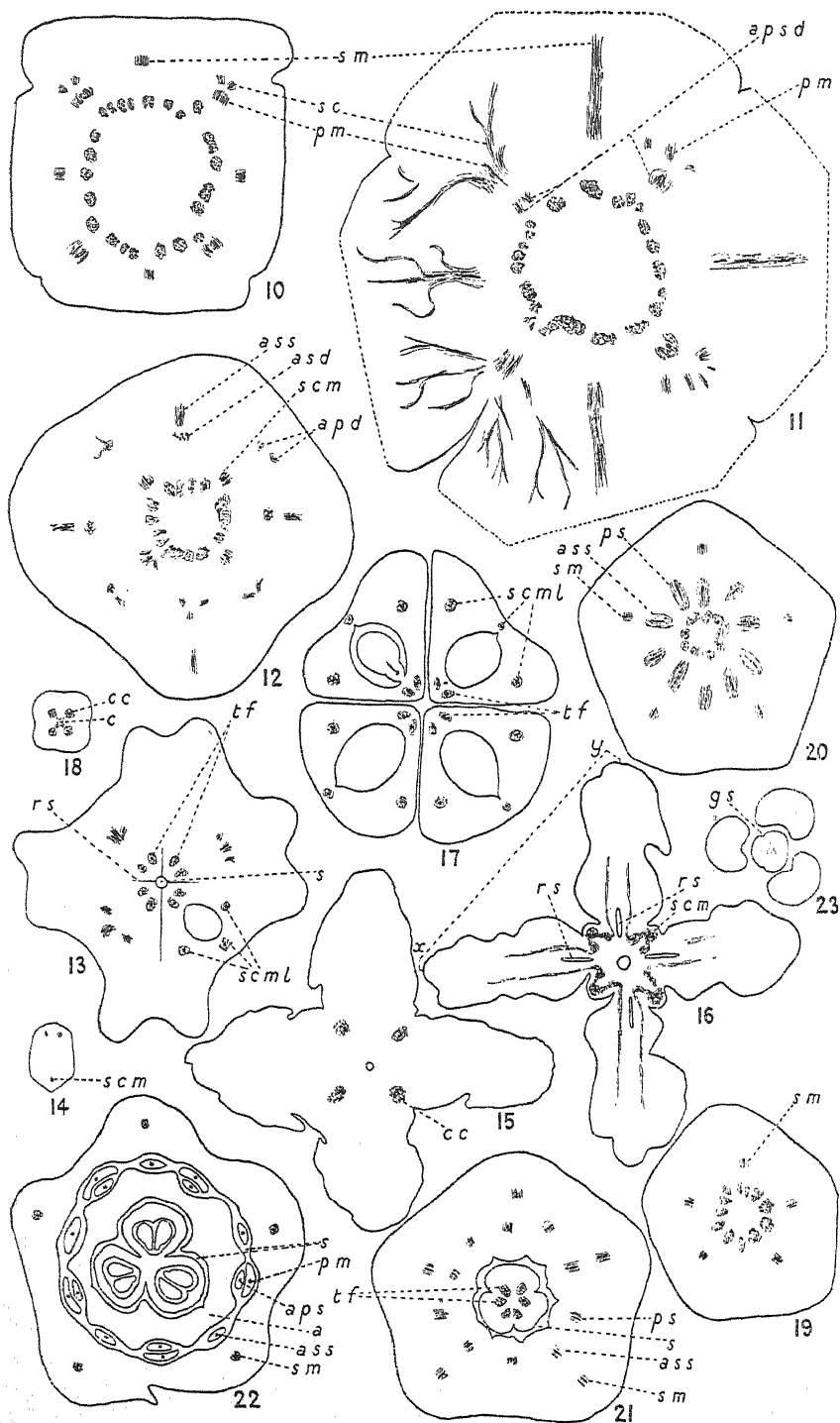
The fact that in the fertile carpels midrib bundles are very generally lacking, or if present, persist only for a short distance, leaves the way open for, if indeed it is not the primary cause of, the radial splitting in the mid-line of these carpels, which brings about the condition of spurious apocarpus characteristic of the majority of rutaceous genera. This splitting may proceed from without inwards, or, where the pith disappears quite early, from the ventral face outwards. In a few types (e.g. *Correa*, *Eriostemon*) it

is initiated by an interstitial slit in the mid-line of the fertile carpel (septum) which may then extend in one or both directions. If the pith persists, extension of the slit inwards does not take place, and the ovary remains slightly lobed but syncarpous, as in *Toddalia*. Such a case as this last-named genus at once suggests comparison with certain types in the Liliaceae, in which family similar slits (septal glands) are characteristic of all but a few types, viz. *Tulipa*, *Fritillaria*, *Lilium*. In these three exceptional genera, fertile as well as sterile carpels possess a well-developed midrib bundle running up in the outer wall of the ovary, and in these types the septal glands are lacking (see 5, p. 163, Figs. 76–80). In other liliaceous genera the single midrib bundle of the fertile carpels is replaced by a double row of radially arranged strands between which the lumen of the gland makes its appearance (*loc. cit.*, Figs. 81–3). In these cases the slit does not extend to the outer surface until near the top of the ovary where the gland opens to the exterior. In rutaceous genera the interstitial slit usually extends to the outer surface at once, hence a condition of more or less complete apocarpy is brought about.

It follows from what has been stated above that the individual ovaries in the pseudo-apocarpous Rutaceae are not constructed of a single carpel, Each is composed of the  $\frac{1}{2}$  +  $\frac{1}{2}$  carpel combination with which we are now familiar in many other families, the whole carpel being the sterile member which is flanked on either side by half a fertile carpel. We see, in fact, brought about prematurely in the rutaceous gynoecium by disjunction the same result which is accomplished by rupture of the tissue in the fruiting stage in all types in which dehiscence is 'septicidal'.

Other facts corroborative of the presence of two carpel whorls in addition to the fundamental feature characteristic of the syncarpous gynoecium in all families (viz. that two successive whorls of midrib bundles on alternate radii are traceable, either being found in types to-day or having obviously been developed in the past and since lost) will be most conveniently dealt with in the descriptions given below of representative genera in which they are to be observed.

As has already been noted, the sterile carpel midribs and loculi in isomerous types are almost invariably situated on the radii of the petals, although where the full number of whorls is present strict alternation of the whorls should bring them in line with the sepals. This disposition is shown in all the types dealt with below to follow directly from the particular vascular scheme in each case. Indeed, a comparison of the mode of origin of the vascular bundles for each successive whorl, whether independent or conjoined with those of a superposed whorl, shows that no better illustration could be found than that afforded by the Rutaceae of the principle laid down in previous accounts, *that it is not alternation between successive whorls of members but alternation between successive whorls of issuing vascular bundles*



FIGS. 10-23. 10-16. *Boronia megastigma* Nees. 10. Base of flower at the level or origin of the sepal midrib bundles in the orthogonal, and of the trunk cords for the sepal commissural marginals and petal midribs in the diagonal, planes. Two of these cords have already split up into their components. Nearer the centre the residual vascular ring in which the early delimitation of the strands which furnish the antepetalous stamen bundles is already to be detected. 11. The same at the level at which dissociation of all four diagonal trunk cords into their components is all but complete. For convenience of arrangement the distal portion of the sepals has been cut away. 12. The flower after exsertion of calyx, corolla, and antesealous stamens, and the emergence of a set of trunk cords on the sepal radii. These later cords have just split up into their stamen and disc components. On the petal radii only the disc strands of the corresponding trunk cords are to be seen, the stamen bundles having passed out into the exserted members. In the centre the residual vascular ring from which the sterile carpel midribs are about to emerge on the diagonal (antepetalous) radii. 13. The ovary still surrounded by disc tissue except in the sector below on the right in which the loculus has made its appearance; here the outer wall is now exposed. The sterile carpel midribs have left the central cylinder and given rise to a pair of lateral veins. In the centre a space resulting from the disappearance of the pith. In a ring around this space the twin bundles of the fertile carpels which are beginning to split from the centre outwards. [Compare Figs. 4-6 showing corresponding stages in *B. heterophylla* where the splitting proceeds at first from without inwards.] In conformity with this splitting the placental strands come to be rearranged in false pairs. 14. Apex of one of the four ovaries, now wholly free through completion of the radial split (spurious apocarpy), showing a sterile carpel midrib and one of the twin strands of the fertile carpel on each side. 15, 16. The four-armed stigma formed by the reunion of the halves of the split carpels, the arms extending outwards on the radii between the loculi, i.e. in line with the sepals. 15. The basal region. Between the arms, i.e. over the loculi, a single vascular cord representing a sterile carpel midrib fused with one of the twin strands of the fertile carpel on each side. 16. The apical region. The vascular cords seen in 15 are breaking up into their components, each fertile carpel strand, after separating from the sterile carpel midrib, curves outwards into the half of the neighbouring arm to which it properly belongs. In the mid-line of each arm a radial split where the reunited halves of the carpel have begun to separate again from each other. (See also Figs. 85 C, D.) 17, 18 *B. fastigiata* Bartl. 17. The gynoeceum of four separate ovaries formed as in *B. heterophylla* (see Figs. 4-6). Each ovary shows a sterile carpel midrib with a pair of laterals and one of the twin strands of the fertile carpel on either side. 18. The style column just before it terminates in four (in the specimen figured) barely confluent stigmas which stand over the loculi and hence in line with the petals. The three vascular strands seen in each ovary wall in 17 have fused into a single cord. (See also Figs. 7, 8.) Four areas of conducting tissue in line with these cords connect with the central canal. 19-23. *Agathosma imbricata* Willd. From a pentamerous flower. 19. Flower base at the level at which the sepal midrib bundles (here the sole vascular supply of the calyx) turn outwards to the periphery. 20. The same after the five trunk cords which furnish the petal midribs and the bundles for the superposed stamens have left the central cylinder on the one set of radii and have been followed on the other set of radii by the five bundles for the antesealous stamens. (The disc strands are not represented). 21. The same at the level of disjunction of the oligomerous gynoeceum from the surrounding ring of perianth-stamen-disc tissue. Radial splitting of the fertile carpels from without inwards has already begun. No midrib bundles are traceable in the carpels. Near the centre the twin placental strands of the three fertile carpels, each pair becoming separated later through extension of the line of cleavage. 22. The flower after the petals and antepetalous stamens have become disjoined. (The vascular system of the gynoeceum is not represented.) 23. The gynoeceum after it has become split into three separate ovaries in which the loculi have become closed. In the centre the 'gynobasic' style column showing a single three-lobed area of conducting tissue formed by the confluence of three originally separate areas (compare Fig. 18).

(by no means necessarily one and the same thing) *which constitutes the fundamental relation underlying all floral ground-plans.* This will be apparent if we now consider the whole vascular scheme in some illustrative cases. [It is to be understood that the full number of whorls is present in the types described unless otherwise stated.]

*Adenandra uniflora* Willd. Calyx, corolla, and androecium are coherent at their base into a thick cup which is lined with disc tissue, the ovary being sessile and sunk in the cup so formed. The sepals are furnished with commissural marginal veins. On the one set of radii the bundles for the antesepalous members of the androecium turn out independently, following those for the sepal midribs. On the alternate radii sepal commissural marginals, petal midribs, and the bundles for the antepetalous members of the androecium all pass out together conjoined into a single set of trunk cords. These are followed later by the set of strands which supply the disc. These strands bring with them to the periphery of the vascular cylinder the vascular elements which initiate the outer whorl of bundles for the gynoecium, i.e. those for the sterile members. The breaking up of this complex takes place in this type at the boundary of the central cylinder instead, as is more usual, in the cortex at some greater or lesser distance from it. The appearance of the sterile carpel bundles on the petal radii is thus in accordance with the general scheme of alternation of the successive sets of simultaneously organized bundles for the several whorls, this position involving, as a natural consequence, antepetalous loculi.

*Agathosma imbricata* Willd. (Figs. 19-23). Here the sepals are without marginal veins. The out-turning of the sepal midrib bundles is followed by the appearance on the same set of radii of a set of trunk cords which give rise to the antesepalous stamen bundles and the disc strands on these radii. On the alternate radii one set of trunk cords furnishes the petal midribs, the bundles for the superposed stamens and the disc strands on these radii. The gynoecium is usually oligomerous, hence the position of the carpels and loculi call for no special comment. Midrib bundles are no longer traceable in either the sterile or the fertile carpels. The gynoecium shows characteristic radial splitting of the fertile members and a 'gynobasic' style filament.

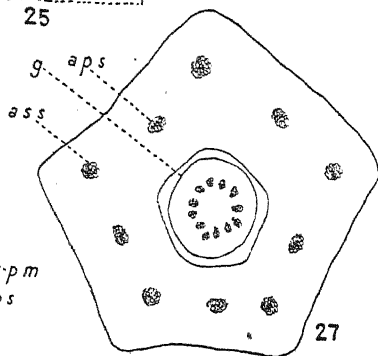
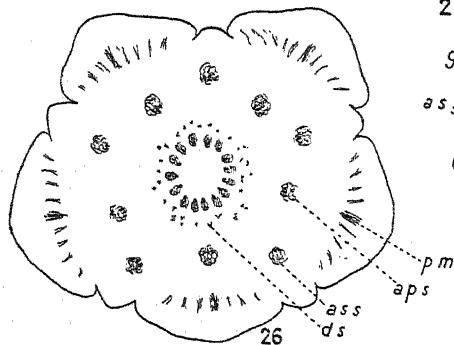
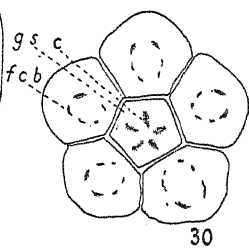
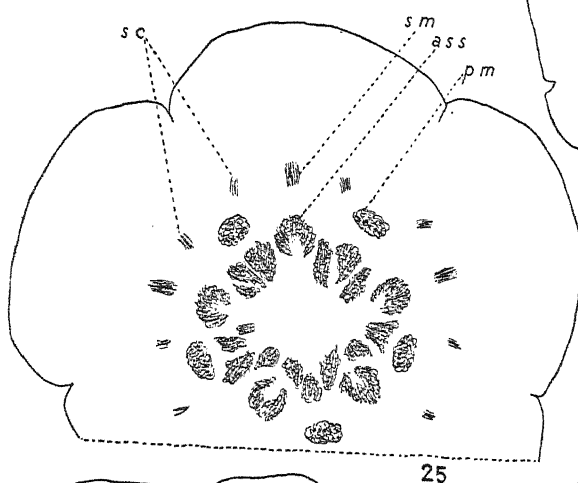
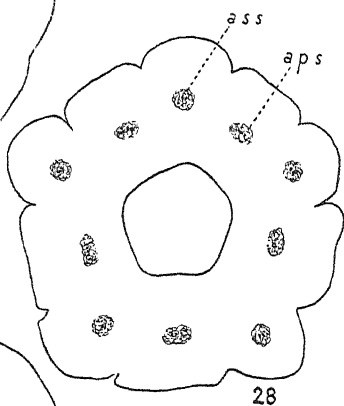
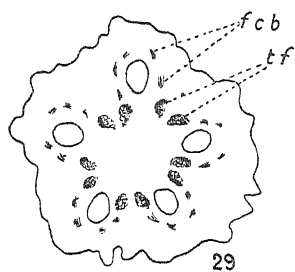
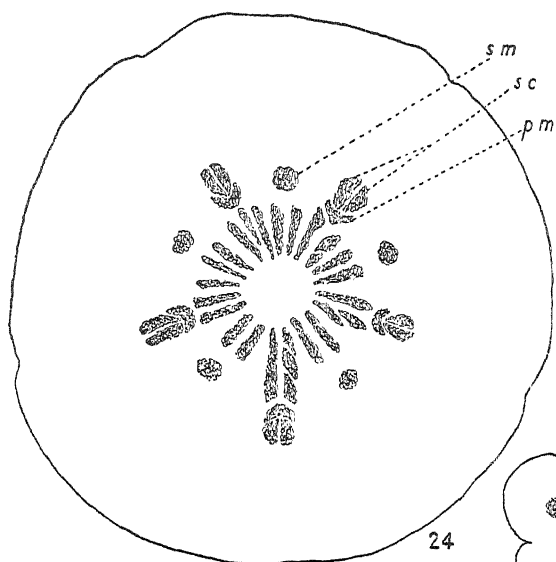
*Barosma crenulata* Hook. (Figs. 31-4). Here, as in *Adenandra*, the antepetalous stamen bundles as well as the petal midribs are conjoined with the sepal commissural marginals into one set of trunk cords, while the antesepalous stamen bundles turn out independently. Disc strands follow on both sets of radii, and are followed in their turn on both sets of radii by a carpel midrib bundle, for in this type we have an exceptional case in which both sets of carpel midribs attain an appreciable degree of differentiation. That the outer (sterile) set should be those on the petal radii is, in the above circumstances, entirely in accord with the normal scheme of

alternation of the several whorls of outgoing bundles. Antepetalous loculi naturally follow.

*Boronia heterophylla* F. Muell. (Figs. 1-9 and 85 B), *B. megastigma* Nees (Figs. 10-16 and 85 C, D), *B. fastigiata* Bartl. (Figs. 17, 18, and 85 A). The out-turning sepal midrib bundles are followed later by an independent set of trunk cords which break up on their way to the periphery, giving rise in each case to a bundle for the corresponding antesepalous stamen and to disc strands. After the emergence of the sepal midrib bundles the vascular elements in the central ring lying on the alternate radii become organized in each case into a complex which slightly projects at each angle as the ring assumes a four-sided outline. Each complex breaks up as it begins to turn outwards. The resulting peripheral portion, which leaves the ring at once, breaks up further into the sepal commissural marginals and a petal midrib; the remaining portion left behind at the angle turns outwards a little later, in its turn breaking up further in each case into the bundle for the corresponding antepetalous stamen and the disc strands originating on this radius. The sepal commissural marginals, petal midribs, antepetalous stamen bundles and disc strands all being derived on each radius from one defined group of vascular elements<sup>1</sup> (although this relation is obscured by the manner of break-up) it is in accord with the general scheme of alteration of the successive whorls of outgoing bundles that the first bundles to turn outwards for the gynoecium should emerge on the petal radii, and hence that the loculi should be antepetalous.

In those species with styles distinct or united only at the apex, each terminating in a small capitate stigma, as in *B. fastigiata* (Fig. 85 A), it is at once evident that each stigma is centred over a locusus and hence is antepetalous. Where the whole gynoecium terminates in a single stout cone surmounted by four small triangular horizontal lobes, as in *B. heterophylla* (Fig. 85 B), the same relation is found to hold, though it is not so easily observed. Each of the four lobes is morphologically equivalent to one of the four capitate stigmas of *fastigiata* and is situated on a corresponding radius. On the other hand, in such species as *megastigma* and *Purdieana* in which the gynoecium terminates in four large, almost sessile, antesepalous, horizontal 'arms' (Fig. 85 C), these 'arms' are *not* comparable morphologically with the individual stigma structures of such species as *fastigiata* and *heterophylla*. On the present interpretation of the gynoecium, each individual stigma in the two last-named species represents the termination of one whole sterile carpel and half the fertile carpel on either side. Now in *megastigma* and similar forms the above-mentioned arms are centred on the radii *between* the loculi. Each is formed, as will appear, of the partially reunited halves of one whole fertile carpel, which at a lower level shows the complete radial split characteristic of all *Boronia* species

<sup>1</sup> Best seen in *B. heterophylla*.





and of the Rutaceae in general. To regard the four arms as the four individual stigmas of the tetramerous gynoecium is to hold them to be commissural. Now it is to be noted that in no single case yet investigated has the commissural stigma proved to be a reality if the conception of sterile and fertile carpels be accepted. Nor is it so here. The boundaries of the real stigma units cut across the four-arm configuration, as is plainly shown by the vascular scheme, each unit consisting, not of one whole arm, but of the sector between two neighbouring arms (= one whole valve carpel) together with half the arm (= half one fertile carpel) on either side. That is to say, each individual stigmatic unit in the *megastigma* type has the same construction and occupies the same position as the stigmatic unit in the *fastigiata* and *heterophylla* types. As in these latter types, it is centred over the loculus and hence is antepetalous. The real point of difference between the two groups of species is that in the *fastigiata-heterophylla* group the sterile carpel slightly exceeds the halved fertile carpels in length. This relation results, when style and stigma units are separate, in the slender filaments and capitate stigmas of *fastigiata* (Fig. 85 A), when conjoined, in the stout cone topped with the flat incurved lobes of *heterophylla* (Fig. 85 B). In the *megastigma* class this relation is reversed, the fertile carpels far exceeding the sterile members in length. But the four component stigma units are still centred over the sterile members. They have the form of four anchor heads, from which the shanks have been cut off, so placed that they are in contact, with the heads all meeting at a common centre. Just as each anchor head shows the top of the shank and the fluke on each side; so the unit components of the whole stigma are formed of the apex of a sterile carpel together with the half of a much longer fertile carpel on each side. The two adjacent flukes of neighbouring anchors correspond with the partially reunited halves of one fertile member, i.e. with one arm of the cross. The arms are, as they

FIGS. 24-30. *Calodendrum capensis* Thunb. 24. Flower base after the five bundles for the sepal midribs have emerged from the central cylinder. On the alternate set of radii the complex which later breaks up into sepal commissural marginals and a petal midrib is already defined and about to turn outwards. 25. The same after the petal midribs have become dissociated from the sepal marginals. The bundles for the antesepalous stamens are about to turn outwards on the alternate radii. [For convenience of arrangement a portion of the two lower sepals has been cut away.] 26. The same after exertion of the calyx. At the periphery the five petals not yet exerted. Nearer the centre the ring of bundles for the two stamen whorls, those for the antesepalous members standing farther out than those for the antepetalous members. Within this ring the scattered disc strands, and within these again the residual vascular bundles serving the gynoecium. 27. The staminal ring lined with disc tissue. The position of the bundles indicates a diplostemonous arrangement. In the centre the now free cylindrical gynophore. 28. The staminal ring still showing a diplostemonous arrangement at the level at which the filaments begin to separate. 29. Ovary base after the appearance of the loculi which stand in line with the sepals. On the alternate radii the twin placental bundles of the fertile carpels. Strands from these bundles pass outwards around the loculi into the outer wall of the ovary. 30. The five ovaries arising by spurious apocarp in a ring round the 'gynobasic' style column. In the style column five styler canals in line with the now-closed loculi. In each ovary two sets of vascular strands are turning inwards on their way to enter the style column. These two sets belong respectively to half the fertile carpel on each side of the intervening sterile member, the midrib bundle of which is not developed.

appear to be, commissural, for they represent an alternate whorl of fertile carpels, but the quadrants corresponding with the four-unit stigmas are not. The boundaries of the quadrants are indicated in a view of the whole stigmatic structure viewed from above shown in Fig. 85 C. A comparison of the vascular scheme in these three species affords complete confirmation of the above interpretation. In *heterophylla* each quadrant of the style-stigma column corresponding with one of the four terminal lobes receives the whole midrib bundle of a sterile carpel, and on each side of this another bundle representing half the vascular system of the neighbouring fertile carpel to right and left respectively (Fig. 7). These three bundles fuse as they pass upward, each quadrant thereafter showing a single strand (Fig. 8). In *megastigma* the grouping of the bundles entering the short style is the same. But here fusion of the sterile carpel midrib with the bundle belonging to half of the fertile carpel on each side (Fig. 15) is followed later by dissociation of the cords thus formed into their three components. This separation is seen in process of taking place in Fig. 16. The diagonal sectors between the arms are formed of the sterile carpels. From the midrib bundles of these carpels the strands proper to half the fertile carpel on each side are turning horizontally outwards and passing into the corresponding half arm. Each of the four arms shows a median radial slit. The radial splitting of the fertile carpels characteristic of the ovary region in *Boronia*, as in the Rutaceae in general, being, as is also characteristic in this family, non-existent in the style region, here reappears at the stigma level as an interstitial slit which extends shortly to the surface. It is thus clear that each arm represents one whole fertile carpel and each sector between the arms one whole sterile carpel. And further, that the four-arm configuration lies crosswise to the boundaries of the four component stigma units, each of which consists, not of one whole arm, but of the whole sector lying between the radial slits of two neighbouring arms (see  $x, y$  in Fig. 8), that is to say, of  $\frac{1}{2} + \frac{1}{2}$  carpels.

Thus in *Boronia*, as in every other case so far investigated, the commissural stigma, a conception needed to cover the facts on the old traditional view that the syncarpous gynoecium is composed of a single whorl of valve carpels, proves to be neither real, nor, on the present interpretation, required.

*Calodendrum capensis* Thunb. (Figs. 24–30). This genus is of exceptional interest, for unlike other isomerous members of the family having the full number of floral whorls, it has a *diplostemonous* flower with the loculi originating in line with the *sepals*. No explanation of these opposite relations among nearly related forms having the same ground-plan has so far been suggested. Indeed, it is possible that the above unusual feature of the *Calodendrum* gynoecium has escaped notice, for I have been unable to find any reference to the orientation of the gynoecium in descriptions of the

flower in systematic works. This conjecture is rendered the more likely by the fact that the ovary here eventually becomes raised on an extremely long gynophore, which, as it elongates, becomes slightly twisted, a feature which tends to obscure the original position of the loculi and the true radial relations of carpel and perianth whorls. The interest attaching to the case of *Calodendrum*, however, goes beyond the fact that it apparently stands alone among comparable Rutaceae in respect of the position of the loculi, for the results of the present series of investigations of many families have led to the conclusion that in the complete Dicotyledon flower *the uninterrupted alternation of all the whorls only occurs when some specific adjustment comes into play which provides a way of overcoming resistance to expansion, or as we may alternatively express it, of relieving the congestion which, it may be supposed, necessarily increases towards the centre as each successive whorl, still surrounded by, and continuous with, the tissue of the earlier whorls, begins to extend outwards.* It is perhaps scarcely realized how rare is the isomerous Dicotyledon showing alternation throughout all whorls of perianth, androecium, and gynoecium. I doubt, indeed, if this condition ever occurs unless some special arrangement is also present affording the necessary 'relief' either in the final stages, as in *Calodendrum* (see below), or at some earlier point, as is well illustrated, e.g. in a member of another family, *Stachyurus praecox* Sieb., and Zucc. (Stachyuraceae).

Furthermore, on the present view we can well understand why the obdiplostemenous condition is not found among Monocotyledons. (*Rapatea*, contrary to description, is no exception.) In the hypogynous, isomerous, trimerous types of the Liliaceae the ground-plan is the same as in the rutaceous genus *Triphasia* and the ericaceous genus *Tripetaleia*, yet in contrast with these two types the liliaceous loculi are antesealous and the stamens diplostemenous. They are so because in the Monocotyledon the conditions which are held to set up a state of congestion do not obtain. There is no central vascular cylinder from which the carpel-midrib bundles originate as in the typical Dicotyledon. Instead, a minimum of vascular tissue is present in the form of widely separated bundles scattered in the parenchymatous tissue. If we take for comparison those liliaceous types in which a bundle is present in the fertile carpels corresponding with the midrib bundle of the sterile members, as, e.g., *Tulipa*, we find (1) that the turning outwards of the carpel midrib bundles preparatory to the expansion of the carpels and the formation of loculi does not take place until after the exertion of the stamens, and (2) that the sterile carpel midribs stand from the outset at such a distance from the axis centre that when they turn outwards they run horizontally for a very short distance before turning upwards. As no bundles lie peripherally to them the way is open. There is no blocking involving compression, consequently no state of congestion is set up. In these circumstances there is no disturbance of the

regular sequence, the androecium is diplostemenous and the loculi antesepalous.

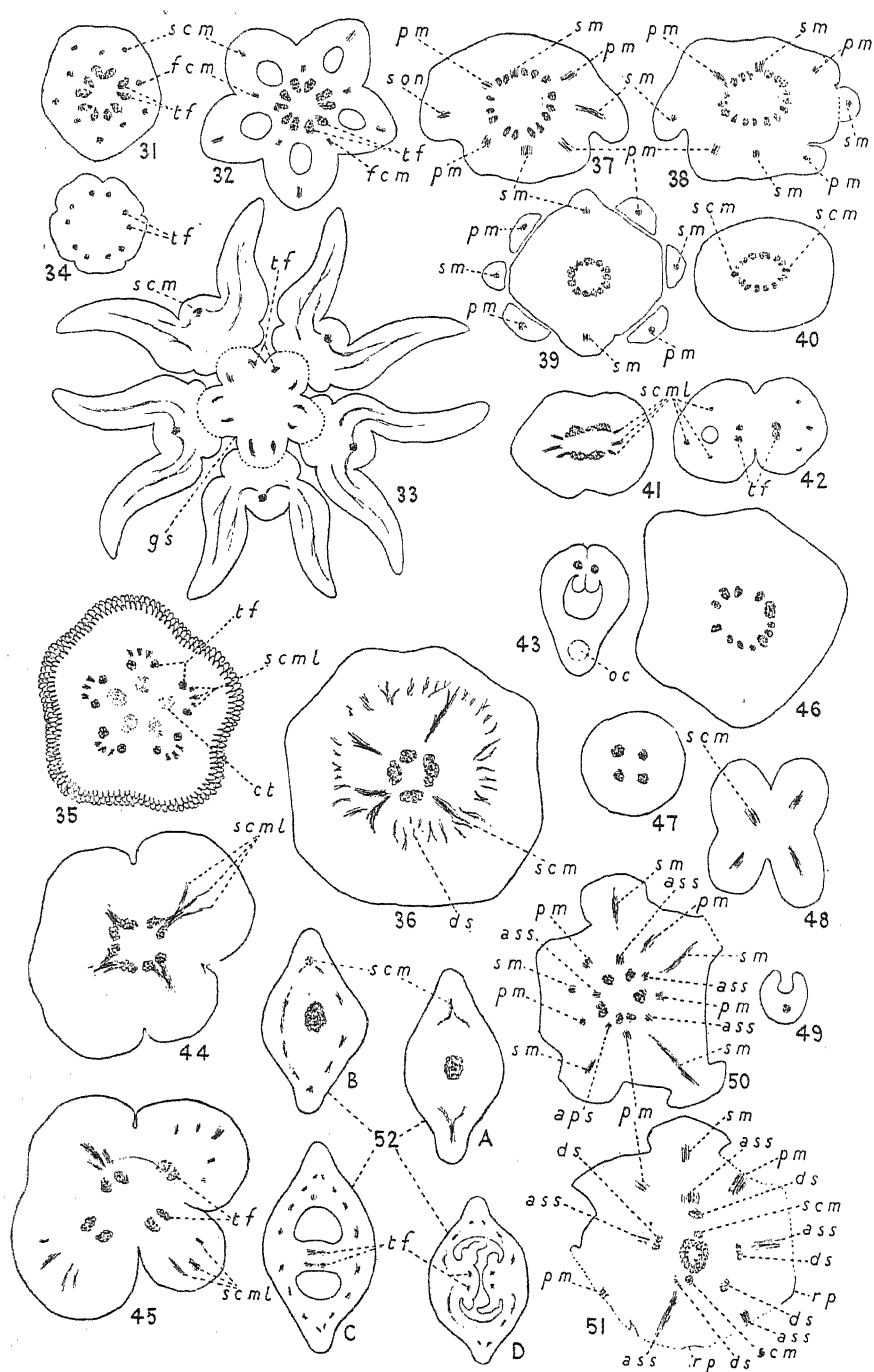
Sections taken through the base of the flower of *Calodendrum* reveal the following facts. The thick sepals which at the base have a convex inner outline are still continuous with each other at their edges and with the axis tissue on their inner face at the level at which the bundles for the corolla and androecium are already defined and lie outside the vascular cylinder. The sepals are furnished with commissural marginals which carry out the petal midrib bundles in the usual way (Fig. 24). The bundles for both stamen whorls leave the central cylinder independently of the bundles for the perianth (Figs. 25, 26). Bundles for the short tubular disc arise on both sets of radii almost simultaneously with the stamen bundles. Here we have a perfectly regular and uninterrupted alternation of the outgoing strands for the successive whorls, which is continued by the development of those for the outer sterile carpels, one result of this alternation being that the loculi make their appearance on the radii of the sepals. Yet here, in particular, the contrary result might have been anticipated. For any 'relief', or gain in time which conceivably results when the petal midrib bundles are carried out with the sepal marginals is neutralized in the present case owing to the fact that the petal bundles become detached at the moment the trunk cords emerge, and are left behind at the boundary of the central cylinder. They are prevented from at once making their way farther out by the fact that the calyx still forms a continuous belt of tissue at the periphery at this level. As a result the antepetalous stamen bundles are in turn kept back and lie farther in (nearer the centre) than those for the antesepalous stamens (Figs. 26-28). How then does it remain possible for the scheme of alternation to be continued by both the carpellary whorls if the present conception of the cause of obdiplostemony and antepetalous loculi, in cases where perianth midribs and stamen bundles turn out independently on their respective radii, is well founded? The explanation is to be found in the fact that the gynophore becomes disjoined from, and lies free within, the encircling staminal tube *below the level at which the carpels begin to extend in the radial direction* (Fig. 27). There is therefore not the thrust from within outwards to which the developing androecium is ordinarily subjected. There is no cumulative congestion effect such as is presumed to occur where there is no break in continuity between the more internal developing gynoeceum tissue and that of the several outer whorls, and no gain in time through fusion of the midrib bundles of two superposed whorls into a single trunk cord. A simpler and more convincing case in support of the view here taken that a causal connexion exists between antepetalous loculi and obdiplostemony could hardly be produced. Where, as here, there is no blocking effect to retard the expanding of the gynoeceum on the sepal radii there is no obdiplostemony. Where, on the

other hand, such blocking exists, as is particularly well illustrated in *Erodium* and *Geranium*, in which the radial dimension of the antepetalous carpels exceeds to an exceptional degree that of the antesepalous members, obdiplostemony is well marked. It is difficult to see in what other way than that here suggested it is possible to account for the change from alternation to superposition of the whorls and at the same time to cover all the facts. As the gynophore in *Calodendrum* elongates, a slight twisting eventually brings the loculi more or less over the petals, consequently unless the flower is examined in the bud stage the true position of the two carpel whorls is not obvious on inspection.

*Dictamnus Fraxinella* Pers. The bundles which turn out from the central cylinder for the sepal midribs are followed immediately by the composite bundles composed of the bundles for the antesepalous stamens and of the strands for the corresponding sectors of the disc. On the other hand, on the alternate radii the vascular components for sepal commissural marginals, petal midribs, antepetalous stamen bundles, and the strands for the corresponding sectors of the disc all leave the central cylinder simultaneously conjoined into one set of trunk cords. Hence it is entirely in accord with the general scheme of alternation of the successive sets of out-turning bundles that the sterile carpel midribs should follow on the petal radii, thus accounting for the antepetalous position of the loculi.

*Diosma succulenta* Berg. A 5+0. In this type the sepals are not supplied with commissural marginal veins and no antepetalous stamen bundles are formed. Two successive whorls of bundles, sepal midribs, and antesepalous stamen bundles turn out on the sepal radii before the emergence of the bundles for the gynoeceium, while only one set of bundles, the petal midribs, emerge on the petal radii. Hence the next whorl of bundles to turn outwards, those of the sterile carpels, in accordance with the general scheme of alternation, emerge on the petal radii.

*Ruta bracteosa* DC., *R. graveolens* L. (Fig. 36). The sepal midribs, which here form their own laterals, are followed on the same radii by the independent bundles of the antesepalous stamens. The petal midribs are similarly followed by the bundles for the antepetalous stamens. Strands for the disc issue from the central cylinder later again, but as they appear to emerge irregularly round the whole circumference of the central cylinder they presumably do not affect the sequence of the succeeding whorls. These strands show a marked degree of degeneration, many remaining entirely undifferentiated; even those that undergo a considerable degree of differentiation usually have the basal region connected with the central cylinder quite unligified. The regular alternation exhibited by the perianth and stamen whorls is broken by the superposition of the sterile carpel midribs upon those of the preceding whorl, i.e. upon the antepetalous stamens, a position which brings the loculi in line with the petals. This is



FIGS. 31-52. 31-4. *Barosma crenulata* Hook. From a pentamerous flower. 31. The gynophore. Towards the periphery on the one set of radii the midrib bundles for the whorl of outer sterile carpels; on the alternate set of radii the corresponding bundles for the inner fertile carpels. Near the centre the twin placental strands of each fertile member. 32. Ovary base immediately after the appearance of the loculi. The vascular scheme as in 31. 33. Ovary apex showing the pair of outgrowths from each sterile carpel supplied by branches from the midrib. In the centre the outline of the 'gynobasic' style column is defined. Within this outline the placental strands of the five fertile carpels grouped in false pairs. 34. The style column with the true pairs of placental strands alternating with the convexities in the outline which indicate the radii of the loculi. 35. *Toddalia aculeata* Pers. ♀. The gynoeceium of an isomerous pentamerous ♀ flower at the stigma level. At the circumference a continuous layer of papillose cells. Towards the periphery, on the radii of the loculi, the five groups of vascular strands belonging to the sterile carpels. Slightly nearer the centre the twin placental strands of the fertile carpels arranged in false pairs, the pairs proper to each carpel member alternating with the five areas of conducting tissue superposed on the loculi. In the centre the persistent pith. (See also Fig. 87.) 36. *Ruta graveolens* L. From a tetramerous flower. The ovary base surrounded by disc tissue. On the diagonal radii the issuing midrib bundles of the four sterile carpels. Between these bundles the branches derived from them and also strands for the disc, both with the basal region frequently undifferentiated. In the centre the residual vascular tissue not yet organized into the twin placental strands of the four fertile members. 37-43. *Zanthoxylum planispinum* Sieb. and Zucc. ♀. From a K 4 C 4 flower with a dimerous gynoeceium. 37, 38. Flower base at the level of origin of the bundles for the perianth members. 39. The same after exertion of six members of the perianth. (Exsertion of the two median sepals is delayed until after that of the petals.) 40. The gynophore. In the central ring the two bundles which will furnish the midribs of the two sterile carpels are already defined. 41. Base of the gynoeceium at the level at which the two sterile carpel midribs, each with a pair of laterals, turn out from the central cylinder. 42. The same after the organization of the residual vascular elements seen in 41 into the twin strands of two median fertile carpels and their later separation into false pairs. These carpels have already begun to split in half from without inwards. 43. One of the two ovaries formed by the radial splitting seen in 42. The two bundles as in Fig. 14. In the position of the sterile midrib which is no longer traceable is a large secretory cavity. 44, 45. *Z. Bungei* Planch. ♀. The gynoeceium of an isomerous tetramerous flower. In 44 the sterile carpel midribs are in process of turning out from the central cylinder, leaving behind the vascular elements which become organized into the twin placental strands of the fertile carpels on the alternate radii. 45. After the organization of the twin strands of the fertile carpels and their separation into false pairs preparatory to the complete splitting in half of these members. 46-9. *Z. fraxineum* Willd., ♂. 46. Flower base above the level of exsertion of the perianth and stamens. At the periphery, disc tissue. In the centre a complete ring of bundles, of which only the four in the diagonal planes show differentiated xylem elements. 47. The gynophore. In the diagonal planes the four differentiated bundles seen in 46 which furnish the midribs of four sterile carpels. 48. The four sterile carpels in process of separation. The midribs are turning outwards preparatory to the attempt to form loculi. 49. A single carpel disjoined from the others at the level at which it passes into the style filament. 50-2. *Ptelea trifoliata* L., ♂. From a pentamerous flower. 50. Flower base just below the level of exsertion of five sepals which are without commissural marginals. On the alternate radii the bundles which pass intact into the petals to furnish the midribs. About to emerge from the central cylinder on the radii of the sepals are five trunk cords which give rise to the bundles for an antesepalous stamen whorl and five groups of strands for the disc. (A portion of one sepal has been cut away.) 51. The same after all but two members of the perianth have been detached. Stamen bundles and disc strands are now disjoined. The bundles for two sterile valve carpel midribs have just turned outwards leaving behind a complete ring of vascular strands. 52 A-D. The gynoeceium now free from the disc. In A branches from the midribs of the two valve carpels are extending round the circumference of the ovary. In B the two branch systems have become continuous, the outer pair of carpels completely enveloping the inner pair of which the vascular bundles are not yet organized. In C the loculi have appeared and the twin placental strands of the two fertile carpels are now differentiated. In D the fertile carpels have become disjoined along their inner face, thus rendering the ovary unilocular. The placentae bear rudimentary ovules.

the arrangement met with\* in most isomerous pentamerous and tetramerous dicotyledon types which have the full number of whorls and in which the bundles for the several whorls turn out independently from the central cylinder. It is one which we may expect to find, on the view outlined above, in all similar cases unless some special feature, such, e.g., as the development of a gynophore which in *Calodendrum* (see above, p. 657) and in certain of the Caryophyllaceae (10, pp. 240 and 242, Fig. 8) affords the necessary relief from what I have described as the cumulative congestion effect which otherwise would be set up, and thus allows the regular alternation seen in the successive whorls of perianth and androecium to be continued in the two whorls of the gynoecium.

*Toddalia aculeata* Pers. ♀. A 5+0 (Figs. 35 and 87). Only the antesealous whorl of the androecium is present. The bundles for these members turn out independently from the central cylinder as in the preceding types. The vascular system of each sterile carpel and of the corresponding sector of the disc is supplied by a group of composite strands which break up immediately after they have emerged into their components. The group belonging to each carpel fails to become consolidated into a midrib bundle, the individual strands pursuing a separate course in the ovary wall. As these groups of bundles turn out next after those for the antesealous stamens, they, and hence also the loculi, naturally occur on the alternate radii, i.e. in line with the petals. The ovary differs from that of the several preceding types in remaining completely syncarpous, the fertile carpels remaining connected by the central parenchyma, which here extends up to the level of the sessile stigma lobes.

*Aurantioideae* (Figs. 56-72 and 86 A-C). This section includes genera in which the gynoecium is syncarpous throughout, the single column formed by the connate styles arising from the summit of the ovary, which has an even unbroken outline in cross-section. In all the four genera examined (*Aegle*, *Citrus*, *Feronia*, and *Triphasia*) the sterile carpel midribs, unlike those in most of the spuriously apocarpous genera, are well developed, while the corresponding bundle of the fertile carpels, as in the apocarpous forms, is lacking, the vascular system of these members consisting only of the twin placental strands and branches derived from them. Certain features distinguishing the individual genera may be briefly noted.

*Feronia elephantum* Correa (Figs. 69-72 and 86 A-C). The vascular bundles for both stamen whorls, which turn out almost simultaneously, arise separately from those of the perianth. Undisturbed by disc development, and surrounding below a very short stipe, and above an ovary with an even circular outline in cross-section, the two whorls come to stand in a single ring, the outer position of the antepetalous anthers seen in the bud stage being merely due to pressure from close packing. The antepetalous position of the sterile carpel midribs, and hence of the loculi, is to be

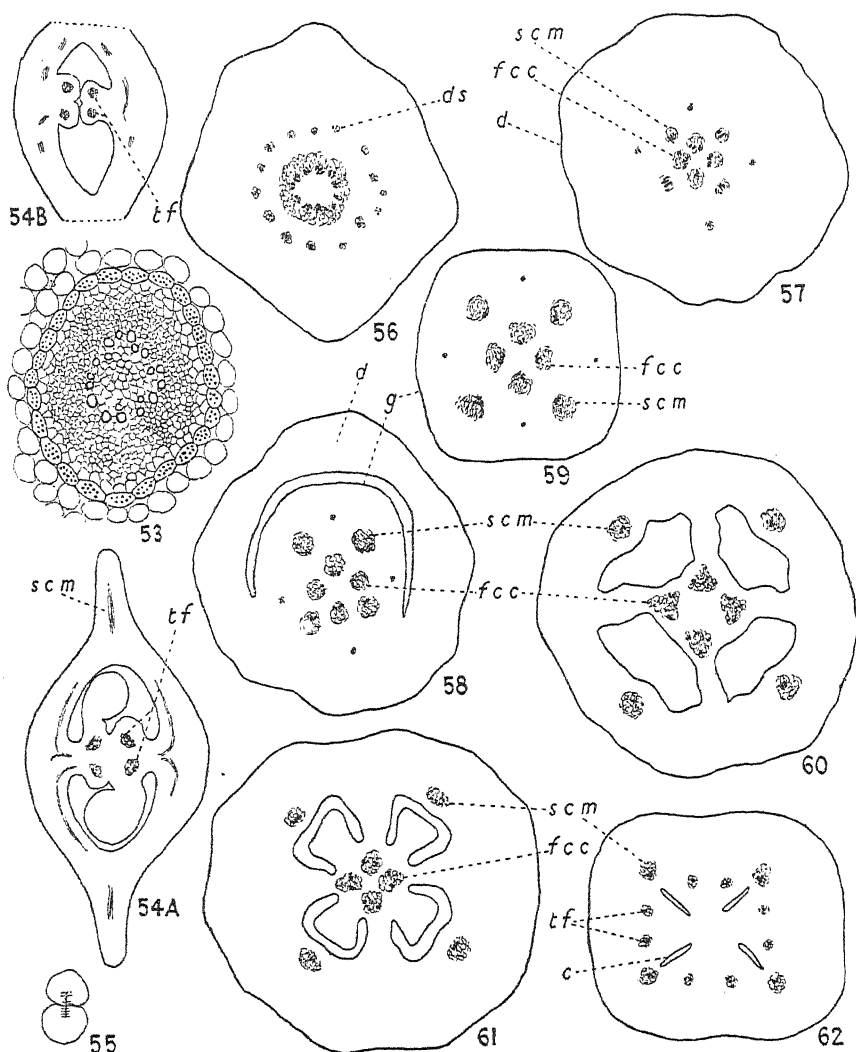


explained as in *Ruta* (see above, p. 659). The disappearance of the pith at the ovary base results in the development of a single loculus and so-called parietal placentation. The stout style and stigma column is formed by the prolongation of both carpel whorls. The stigma lobes are centred over the loculi, each lobe representing a whole sterile carpel with half a fertile carpel on each side. Each is supplied with two bundles, belonging respectively to the fertile half-carpeles, the bundle of the sterile carpel being no longer traceable at the extreme apex.

In *Aegle*, *Citrus*, and *Triphasia* the bundles for the stamens, as in *Feronia*, turn outwards independently of those for the perianth, but here the persistence of the pith results in the multilocular ovary and so-called axile placentation.

*Aegle sepiaria* DC. (Figs. 63, 64). The bundles for the antesealous stamens pass intact into each corresponding member, but those on the antepetalous radii usually branch on their way outwards so that a group of stamens, varying in number, alternate with the single antesealous members. The gynoecium shows the usual arrangement of outer sterile, and alternate inner fertile carpels, but as both sets of members are ordinarily more than 5 and less than 10 in number, their relation to the sepal and petal radii varies in different flowers. The sterile carpel midribs come to an end below the terminal discoid stigma, but the twin placental strands of each fertile member, after coalescing into a single bundle, persist to the apex, so that a cross-section through this region shows the single bundle of a fertile carpel alternating with the slit-like canals which stand over the loculi and hence in line with the sterile carpels.

*Citrus decumana* Murr. (Figs. 65–8). The precise radial relations of stamens and carpels to the perianth whorls are again somewhat complex owing to the branching of the strands which supply the  $\alpha$ -stamens and to the development of twice as many members in each carpel whorl as in either perianth whorl. The construction of the gynoecium is similar to that of *Aegle*, but the twin placental strands of each fertile member here remain distinct to the apex, so that in a cross-section through the stigma region two bundles alternate with each of the stylar canals which are superposed on, and connect with, the loculi. An abnormality long known in the subspecies *Limonum*, cf. *Citrus medica* L., has given rise to the variety *digitata* Risso. This form has more than the interest of a mere curiosity. It has a fundamental significance. In this variety the gynoecium is composed of a ring of tapering sterile carpels which are disjoined almost from the base, and so appear as a ring of finger-shaped structures. There are no loculi and no ovules, no septa, no pulp, no core. There can be little doubt that in this variety the fertile carpels fail to develop, and that the sterile carpels alone are unable to remain conjoined. A more striking piece of evidence in proof of the correctness of the interpretation of the



FIGS. 53-62. 53-5. *Ptelea trifoliata* L. ♀. 53. The vascular cylinder with bundle (starch) sheath and surrounding parenchyma from the region above the level at which the sterile carpel midribs turn outwards and below that at which the bundles for the fertile carpels become organized, the residual xylem elements for these latter bundles being still scattered in a ring round the pith. 54 A, B. The gynoecium. A from the middle bilocular region. In the centre the twin bundles of the two fertile carpels. B. From the upper, unilocular region (compare Figs. 52 C, D). [For convenience of arrangement the 'wings' of the ovary have been cut away.] 55. The two stigma lobes, which are centred over the loculi, after the vascular bundles have come to an end and as the two areas of conducting tissue open out to the surface. 56-62. *Triphasia trifoliata* DC. From a tetramerous flower. 56. The gynophore surrounded by disc tissue immediately above the level of exertion of the perianth and androecium. Towards the periphery some of the bundles supplying the disc. In the centre the residual vascular ring which serves the gynoecium. 57-9. Stages immediately preceding, coincident with, and succeeding disjunction of the disc from the gynophore. The elements of the central vascular ring have become differentiated into four outer diagonal bundles = the midribs of four sterile carpels, and four inner orthogonal bundles = the placental cords of four fertile carpels. Further from the centre are four feebly developed strands more or less in line with

syncarpous gynoecium as conceived on the theory of carpel polymorphism could hardly be forthcoming. This extreme case serves as a clue to certain intermediate conditions in other types (see pp. 666, 671 and Figs. 48-9, 82-4, 88).

*Triphasia trifoliata* DC. (Figs. 56-62). In this species the flower is exceptional in being typically trimerous throughout.<sup>1</sup> The bundles for the sepals (which show true lateral branching), petals, antesepalous and antepetalous stamens arise independently direct from the central vascular cylinder. The six stamens come to stand in a single ring. The strands supplying the disc, which lies within the stamen ring, arise at points round the whole circumference of the vascular cylinder. As disc and gynoecium become disjoined, the line of separation may occur on a circle which leaves some two or three of the innermost of these strands on the central side of the split; these strands then pass up in the ovary wall, one such strand appearing regularly to take up a position on the radius of each fertile carpel. As in the three preceding types, the stigma lobes are centred over the loculi. Here the sterile as well as the fertile carpel bundles persist to the apex, each lobe being supplied with three bundles, the sterile carpel midrib and one of the twin bundles of the fertile carpel on each side. The antepetalous position of the sterile carpel midribs (and hence of the loculi) is ascribed to the same cause as in the comparable case of *Feronia* (see above). Eichler (3, ii, p. 325) asserts that the loculi are antesepalous, and cites Baillon (1, p. 152) in support of this view, while regarding Payer's statement (4, i, p. 113) that they are antepetalous as erroneous. But the position of the stigma lobes is undoubtedly antepetalous, and the course of the bundles confirms Payer's account that the carpels (i.e. on the present interpretation, the outer sterile members) and loculi stand in line with the petals. Confirmation of this view is afforded by the fact that Baillon states definitely in his later description of *Limonia* (including *Triphasia*) (2, iv, p. 406) that the loculi are opposite the petals (*loc. cit.*, p. 493). We may conclude that his earlier statement was either an oversight or was found to be erroneous and was corrected in his later work. Were this not so some comment would assuredly have been made on a relation contrary to that characteristic of other Rutaceae.

The four genera next to be considered are all characterized by an oligomeric gynoecium, though they differ widely in other respects.

the placental cords. (The morphological equivalence of these latter strands is not entirely clear, see p. 647.) 60. The ovary after the appearance of the loculi. The weak bundles seen in the three preceding figures are no longer to be traced. 61. The same after development of the placentae. Each fertile carpel cord supplies the adjacent half placenta bordering the loculus to right and left. 62. The style column. At the angles four slit-like stylar canals in line with the loculi. The vascular cord of each fertile carpel has divided in two in preparation for the formation of the four stigmatic lobes centred over the sterile carpels.

<sup>1</sup> Though the specimen here figured happened to be tetrameric.

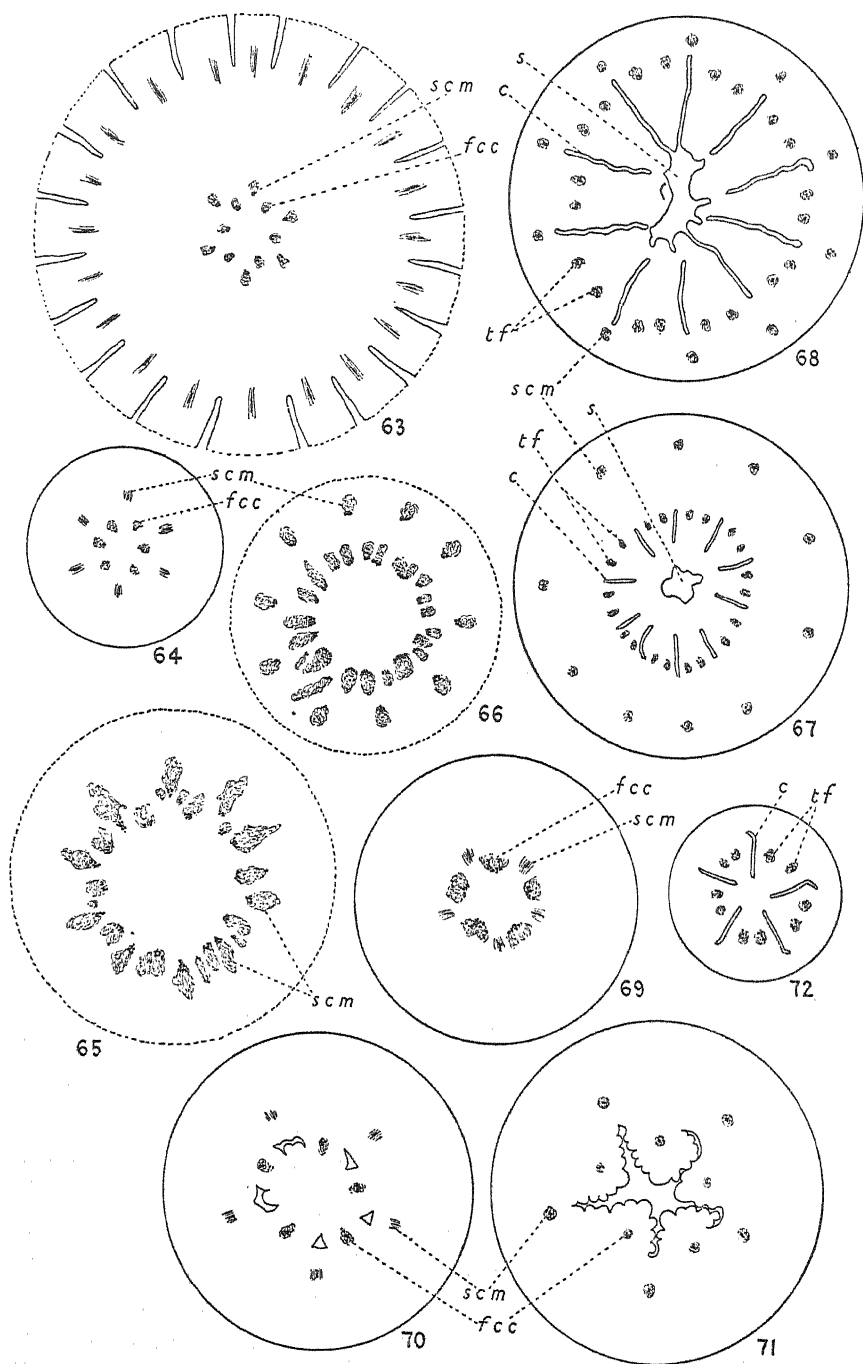
*Zanthoxylum* (Figs. 37-49). The flowers are functionally unisexual, but the male, which have only one staminal whorl, develop a gynoeceium. Marginal veins are lacking in the sepals. Comparison of the construction of the gynoeceium in the male and female flowers confirms the view advanced at an earlier point in these studies that the infertility of the gynoeceium in the male flowers of many dioecious types, functionally though not morphologically unisexual, is due to the suppression of the inner fertile carpel whorl, the outer sterile whorl of valve carpels alone being present. This is well shown in *Z. fraxineum* Willd. After exsertion of the perianth and stamens the residual vascular tissue is seen in cross-section as a complete cylinder of numerous strands, some with xylem elements and some undifferentiated (Fig. 46). Those with xylem elements turn out later to form the midrib bundles of the sterile valve carpels (Fig. 48). Those without shortly come to an end (Figs. 46, 47), whereas in the female flower the strands not utilized in the development of the valve carpels become organized into the bundles of the fertile carpels (Figs. 40-2, 44, 45). Owing to the suppression of the fertile carpels the gynoeceium in these male flowers shows neither placenta nor ovules. In this respect *Zanthoxylum* presents a more extreme case than *Ptelea*, in which both sets of carpels, placentae, and ovules are present in the infertile flowers of the male, but the latter structures are only imperfectly developed (see below). If a locus is formed it is a mere slit which immediately opens to the exterior (see Fig. 49). In the female flowers complete radial splitting of the fertile carpels, disappearance of the central parenchyma below the level of origin of the styles, and the non-reunion of styles and stigmas leads to a condition of complete (though spurious) apocarpy. The gynoeceium of *Z. planispinum* Sieb. and Zucc. affords an excellent illustration of association of a reduced vascular system with the presence of numerous secretory cavities (see above, p. 648), the midrib bundle of the sterile carpels coming to an end part way up the ovary wall where a large oil cavity makes its appearance (Fig. 43).

*Ptelea trifoliata* L. (Figs. 50-5). Flowers functionally but not morphologically unisexual, A, usually 4-5, occasionally +4-5. The vascular scheme of perianth and androeceium is here of the simplest type. Secondary veins in the sepals are derived by branching from the midrib. The antepetalous members of the androeceium, with bundles emerging, as usually in this family, independently from the central cylinder, are present in the female as well as the male flowers, but the antepetalous whorl is always in the former, and generally also in the latter, suppressed. The ovary in the male flower shows the same scheme of construction as that of the female, but is less well developed. Both exhibit certain unusual features of particular interest from the present point of view. The bundles emerging from the central cylinder to furnish the midribs of the (usually) two sterile carpels are strongly developed, giving rise to a copious system of hori-

zontal lateral veins. These systems, as they extend, become continuous. The outer wall of the ovary is thus shown to be composed wholly of the sterile carpels which completely enclose the fertile members. Herein we find the explanation of the feature, unusual in this family but characteristic of *Ptelea*, of a dry fruit remaining syncarpous and indehiscent. Dehiscence by separation of the sterile carpels is precluded, since the whole vascular scheme of these members constitutes a single closed system. The spurious form of apocarpy found in so many rutaceous types is rendered equally impracticable since the fertile members are completely surrounded. Another feature which is particularly striking in the female flower is the presence of a well-marked bundle (starch) sheath round the central cylinder in the region above the level of emergence of the sterile carpel midribs and below that at which the fertile carpel bundles become organized (see Fig. 53). This fact appears to me to be fatal to the old traditional view that the gynoecium is wholly composed of two valve carpels, since on this interpretation there would appear to be an internode between the level of organization of the midrib and of the marginal veins of the carpellary members.

*Cneoridium dumosum* Hook. f. (Figs. 73-6). G typically 1 + 1.<sup>1</sup> The sepals show a well-developed secondary vascular system derived from true lateral branching of the midribs, supplemented in some members by commissural strands. The bundles for the two stamen whorls arise as in most Rutaceae, those for the antesepalous members turning out independently, those for the antepetalous whorl in conjunction with the petal midribs, which here becomes disjoined close to the boundary of the central cylinder. The gynoecium, hitherto regarded as consisting in typical flowers of a single carpel, is held (in accord with the theory of carpel polymorphism) to be composed of two carpels, one sterile, forming the bulk of the wall of the ovary, one fertile and prolonged above into the 'gynobasic' style filament. A midrib bundle is present in the sterile carpel, but is lacking in the fertile member, which shows only twin fertile bundles and the branches derived from them. The 'gynobasic' style filament springs from one side of the ovary near the base. In cross-section it shows two vascular strands until near the stigma level, where the two fuse before coming to an end. These two strands are formed by the continuation of the twin bundles of the fertile carpel with which the marginal veins of the sterile member have previously become fused. The fertile carpel shows characteristic radial splitting, the cleavage extending to the loculus at a point immediately below the origin of the style filament, the passage thus formed becoming closed again immediately above this point, leaving, however, an

<sup>1</sup> Exceptionally four carpels may develop (two sterile, two fertile). In this case the relations are exactly similar to those described for *Thamnosma montanum* (see later p. 669), in which this is the normal condition.

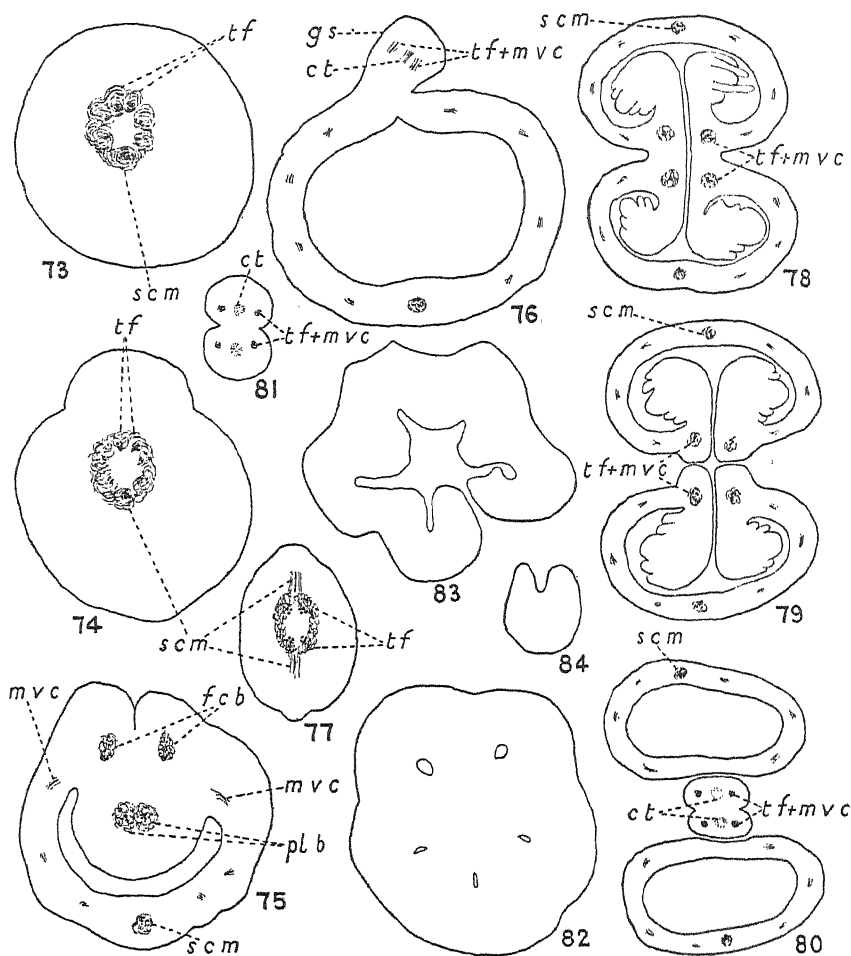


FIGS. 63-72. 63, 64 *Aegle septiaria* DC. 63. The flower above the level of exertion of the perianth. At the periphery the bases of twenty stamens with their bundles cut obliquely. In the

external furrow which is continued up the filament to the stigma level, where the core of conducting tissue extends to the surface. The appearance presented by a cross-section of the ovary below the level of origin of the style filament recalls that seen in many leguminous genera, where also through radial splitting of the fertile carpel, facilitated, if not caused, as already explained, by the absence of a midrib bundle, the loculus is brought into connexion for a short distance with the exterior. The external appearance of the whole *Cneoridium* gynoecium also presents a close resemblance to that of various members of the Phytolaccaceae (section Rivineae), in which the style filament is similarly 'gynobasic'. Indeed, a figure of the gynoecium of *Cneoridium* might well serve to represent that of *Rivina humilis*. There exists, however, though masked by this superficial likeness, a very striking and important distinction between the two genera in respect of the carpel-style relations. This is brought to light by a comparison of the two vascular schemes. In *Rivina* and its allies the fertile carpel comes to an end at the level at which the ovule springs, the sterile carpel alone developing further to enclose the ovule and form the style filament, which shows a single vascular strand (see 8, p. 90, Fig. 24). In *Cneoridium* the fertile carpel is prolonged to the stigma level, the style receiving its two fertile strands with which are fused the two marginal veins of the sterile carpel. In this latter respect, as well as in the radial splitting of the fertile carpel, *Cneoridium* presents a precise parallel with the Leguminosae. Although in this genus the sterile carpel midrib comes prematurely to an end, giving place to a secretory cavity, it is evident from the above-mentioned fusion of bundles, as well as from analogy with other rutaceous genera, that the sterile carpel also contributes to the formation of the style filament, as it does in the Leguminosae.

*Thamnosma montanum* Torr. and Frém. (Figs. 77–81). The gynoecium of *Thamnosma* is interpreted on the same lines as that of *Cneoridium*, except that here there are typically two sterile and two fertile carpels instead of one of each kind, this numerical increase necessarily involving certain differences in the interrelations of the two carpel types. After the

centre the bundles for the two carpel whorls are already differentiated. 64. The gynophore. 65–8. *Citrus decumana* Murr. 65. The central vascular cylinder and surrounding ground tissue immediately above the level of exertion of perianth and androecium. The ten bundles for the ten sterile carpel midribs are differentiated and about to turn outwards. 66. The same after the sterile carpel bundles seen in 65 have left the central cylinder, but before those for the fertile members are yet organized. 67, 68. The style column. Towards the periphery the sterile carpel midribs. Nearer the centre on each alternate radius the twin bundles of a fertile member. In line with each sterile carpel midrib a slit-like styler canal. In the centre the space into which the individual canals eventually open (see 68). 69–72. *Feronia elephantum* Correa. 69. The gynophore. The bundles for the five sterile carpels are beginning to turn outwards. On the alternate radii the placental cords of the five fertile members. 70. Ovary base at the level of origin of the loculi. The vascular scheme as in 69. 71. The ovary now unilocular. 72. The style column. The sterile carpel bundles are no longer to be distinguished. The loculi have contracted to five styler canals. On the alternate radii the twin placental strands of the fertile carpels (see also Figs. 86 A–C).



FIGS. 73-84. 73-6. *Cneoridium dumosum* Hook. 73. The gynoecium. In the centre the vascular cylinder in which the bundles for the sterile carpel midrib and its laterals and the twin bundles for the fertile carpel are already recognizable. 74. Ovary base now beginning to assume the outline of the figure 8. The vascular system as in 73. 75. The ovary after the appearance of the loculus. Below, the sterile carpel with midrib and laterals. Above, the fertile carpel beginning to show characteristic radial splitting with twin bundles from which the strands for the ovules are derived. 76. Upper region of the ovary at the level of the origin of the 'gynobasic' style filament which is seen cut obliquely. In the centre of the filament a tract of conducting tissue, and on either side a bundle of the fertile carpel with which is conjoined the adjacent marginal vein of the sterile carpel. 77-81. *Thamnosma montanum* Torr. and Frém. The gynoecium at the level of origin of the two sterile carpel midrib bundles. 78. The same at the level of transition from the bilocular to the unilocular condition through the complete separation of the two fertile carpels along their inner face. These carpels show the characteristic radial splitting from without inwards. 79. The same after the splitting in half of the two fertile carpels is complete, resulting in the formation of two ovaries (spurious apocarpy) each composed of a sterile carpel flanked by half the neighbouring fertile carpel on each side. 80. The same after the 'gynobasic' style filament has become free. The latter arise by fusion of two filaments each of  $\frac{1}{2} \times \frac{1}{2}$  carpels. 81. The two stigma lobes which are centred over the loculi. 82-4. *Phellodendron japonicum* Maxim. ♂. 82. The gynoecium with very small loculi but without placentae, ovules, or vascular bundles. 83. Upper region of the same now unilocular through disappearance of the central parenchyma at the level of origin of the style filaments which are centred over the loculi. 84. One of the style filaments now free (see also Fig. 88).



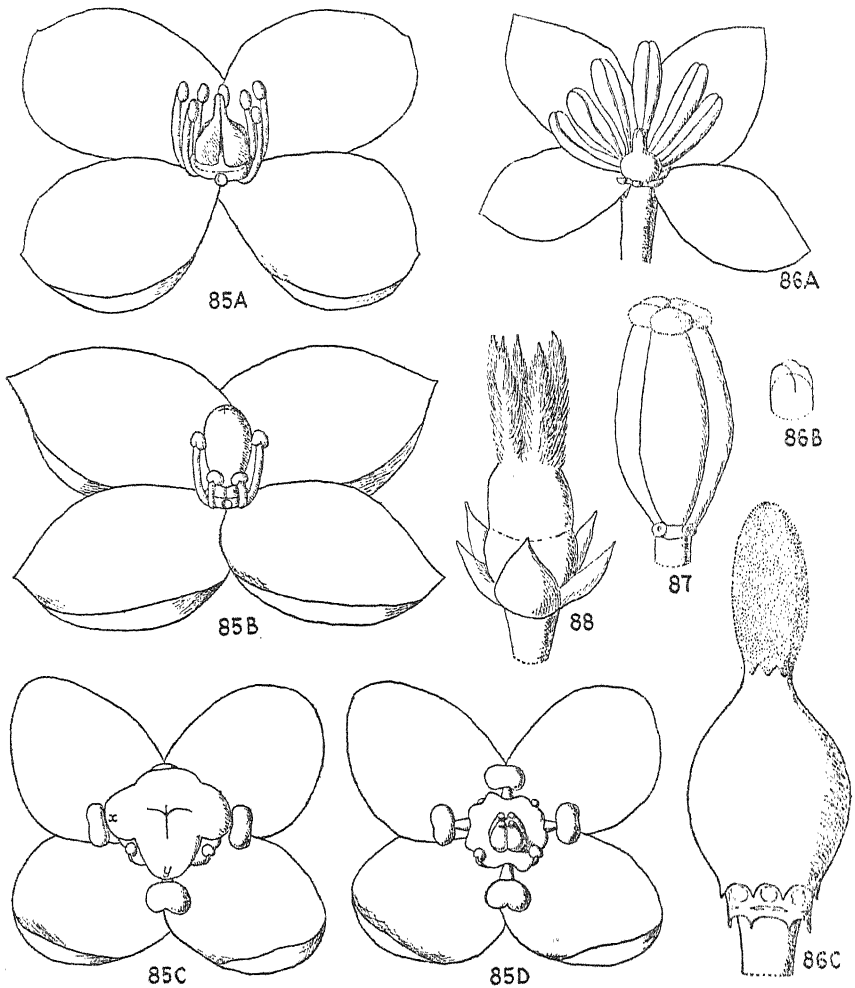
emergence of the sterile carpel midribs from the central cylinder, the appearance of the loculi, and later the disappearance of the pith, the two fertile carpels separate from one another completely along the line of their inner face, the two loculi becoming continuous at this level through the passage so formed. Immediately above this level the radial splitting of the fertile carpels at right angles to the line of their separation, already in progress, becomes complete, giving rise to two separate ovaries. From each springs a 'gynobasic' style filament showing two vascular strands as in *Cneoridium*. Each ovary up to the level of origin of the style is formed of  $\frac{1}{2} \times \frac{1}{2}$  carpels, but here the two half-carpels belong to different fertile members, whereas in *Cneoridium*, where only one fertile carpel is present, they both belong to the same member. Reunion of the two  $\frac{1}{2} \times \frac{1}{2}$  carpel groups follows, the two filaments shortly becoming connate and terminating above in two stigma lobes standing in the same plane as the two sterile carpels.

The two remaining genera to be considered in detail are dioecious types with an isomerous gynoeceium.

*Phellodendron japonicum* Maxim. ♂ (Figs. 82-4, and 88). The level of development attained in the male flower in *Phellodendron* is distinctly lower than that reached in *Ptelea* and *Zanthoxylum* (see above, pp. 666, 667). In a pentamerous male flower of the above species the cylindrical gynophore shows a ring of five vascular strands with differentiated xylem elements in line with the five prominences in the outline which indicate the radii of the five sterile carpels. These bundles (= the sterile carpel midribs) end very shortly; at the level at which the small slit-like loculi make their appearance they are no longer traceable. There are no fertile bundles, no placentae, no ovules, no tracts of conducting tissue. As the pith comes to an end the slit-like loculi become continuous and open above to the exterior. The sterile carpels, unconnected by alternate fertile members, suffer disjunction and continue upwards as the separate tomentose style filaments.

In the female flowers of this and other species (*P. amurense* Rupr., *P. chinense* C. K. Schneider, *P. sachalinense* Rupr.) the styles are connate, the syncarpous ovary being prolonged at its summit into a short column which terminates in a depressed, lobed stigma, the whole gynoeceium being glabrous throughout. The difference in appearance between the gynoeceium of the two sexes is indeed so great that if indisputable evidence to the contrary were not available, the two organs might well be taken to belong to different species. The characteristics of the gynoeceium in the male flower arise from the same cause as the peculiar 'digitate' form of gynoeceium in *Citrus* (see above, p. 663). It is clear that in the male *Phellodendron* flower the sterile carpels are present, but the fertile members are not developed. The vascular elements, which should form the fertile bundles,

remain undifferentiated and soon cease. The corresponding carpels do not develop and the sterile members are unable to remain united by their



FIGS. 85-8. 85 A-D. *Boronia* spp. The flower seen from the front and obliquely from above. A. *B. fastigiata* Bartl. B. *B. heterophylla* F. Muell. C. D. *B. megastigma* Nees. C. The complete flower. D. The same flower after removal of the cross-shaped stigma showing the separate ovaries (spurious apocarpy) and the crenulate disc. 86 A-C. *Feronia elephantum* Correa. A. The flower after removal of the front petal and four stamens in order to expose the gynoeceum. B. Apex of the style column taken from a bud (more highly magnified). C. A young fruit. 87. *Toddalia aculeata* Pers. ♀. The ovary with sessile stigma lobes. 88. *Phellodendron japonicum* Maxim. ♂. Calyx and ovary with separate style filaments.

edges, hence the cavity open above and the separate style filaments in place of the closed ovary and single style column of the female flower.

*Evodia hupehensis* Dode. The gynoecea of the male and female flowers of *Evodia* differ in the same way as in *Phellodendron*, but the con-

trast in the present type is even greater owing to the fact that whereas the several style filaments in the male flower naturally spring from the top of the ovary, those of the female flower are connate and 'gynobasic', a position due to the unequal growth relations of the sterile and fertile members.

Although, owing to lack of suitable material, this interpretation could not be put to the proof in another genus, *Vepres*, there can be no doubt that the different appearance of the gynoeceum in the male and female flowers here is also explicable in the same way. In the male the sterile carpels soon become disjoined, each tapering above into a slender style filament; in the female the ovary passes into a scarcely appreciable, thick style column, terminated in an expanded stigmatic disc.

In these three dioecious genera, as in *Zanthoxylum* (see above, p. 266), we have a most striking confirmation of the theory of carpel polymorphism. On the old traditional view the difference between the male and female flowers of the above genera remains an unexplained anomaly. On the present interpretation the explanation stares one in the face. How else, indeed, is it possible to interpret the most instructive, perhaps, of all cases of this nature, viz. that of species of *Paepalanthus* (e.g. *P. vellosioides* Koern) in which the ovary of the male flower has three styles of precisely the same form as the three non-functional styles of the female flower but lack the other functional three of different form situated on the alternate radii, which are present in female flowers?

#### Zygophyllaceae (Figs. 89–131).

In general the relations of the floral whorls, the position (diplostemenous or obdiplostemenous) of the stamens, the structure of the gynoeceum and position of the loculi in the Zygophyllaceae are explicable on the same lines as in the Rutaceae. It will therefore suffice to supplement the legends to the accompanying figures by a few additional comments. In the genera investigated *Zygophyllum*, *Tribulus*, *Peganum*, *Guaiacum*, *Augea*) the sepals are furnished with commissural marginal veins. The abundant oil-containing cavities of the Rutaceae are lacking and, associated with this difference, is a markedly greater development of the vascular system of the gynoeceum, a midrib bundle with lateral branches or an equivalent system (see later, p. 679) being developed in the sterile carpels of the several above-mentioned types except in *Augea* which, though without oil cavities, has a rich mucilage content. The gynoeceum remains syncarpous throughout, with the style column springing from the summit of the ovary and the central parenchyma persisting, often to the region of the stigma. The antepetalous stamen bundles are usually carried out, as in the Rutaceae, conjoined with the petal midribs and sepal commissural marginals. Those for the antesepalous stamens sometimes turn out independently



(*Zygophyllum sessilifolium* L.), sometimes conjoined with the outgoing sepal midrib bundles (*Z. Fabago* L.). In the latter case dissociation occurs, notwithstanding, at a point on the sepal radii farther from the periphery than the point at which a corresponding dissociation takes place of the trunk cords on the petal radii. Hence in both species the antepetalous stamen bundles maintain a slightly more peripheral position until both whorls are in due course exerted. Closely packed around a gynophore or the cylindrical base of the ovary the two whorls form a single ring, but as the ovary assumes its characteristic five-angled outline an obdiplostemenous condition results.

Unusual features in two of the species investigated, *Zygophyllum sessilifolium* L., and *Peganum Harmala* L., call for more detailed consideration.

In *Zygophyllum sessilifolium* (Figs. 97–106) the whole of the vascular elements from which the bundles for the twenty members of the perianth and androecium (K 5, C 5, A 5, + 5) are derived leave the central cylinder as *one* set of trunk cords which are equidistant but of unequal size. Two of these cords are small; each furnishes the midrib for the sepal on the corresponding radius and the bundle for the superposed stamen. Of the remaining cords two are of large, and one is of intermediate size. Each of these three cords consists not merely of superposed bundles belonging to a *single radius* but of those for *two or more radii*. Each furnishes, in addition to a sepal midrib and antepetalous stamen bundle of the corresponding radius, the commissural sepal marginals, petal midrib and antepetalous stamen bundle belonging to the adjacent radius on one, or on both sides. As these cords make their way to the periphery they become dissociated into their components which take up their proper position. Such collateral fusion, not merely of the midrib bundles of neighbouring perianth members, but also of those belonging to different whorls, recalls the similar appearance to be seen in certain genera of the Leguminosae (as e.g. *Oxytropis*, see 7, p. 246, Fig. 66). In the present case association into trunk cords of the bundles for both neighbouring and superposed members has probably reached the limit for a hypogynous pentamerous type (five trunk cords for all the members of four whorls). At the same time, owing to the fact that

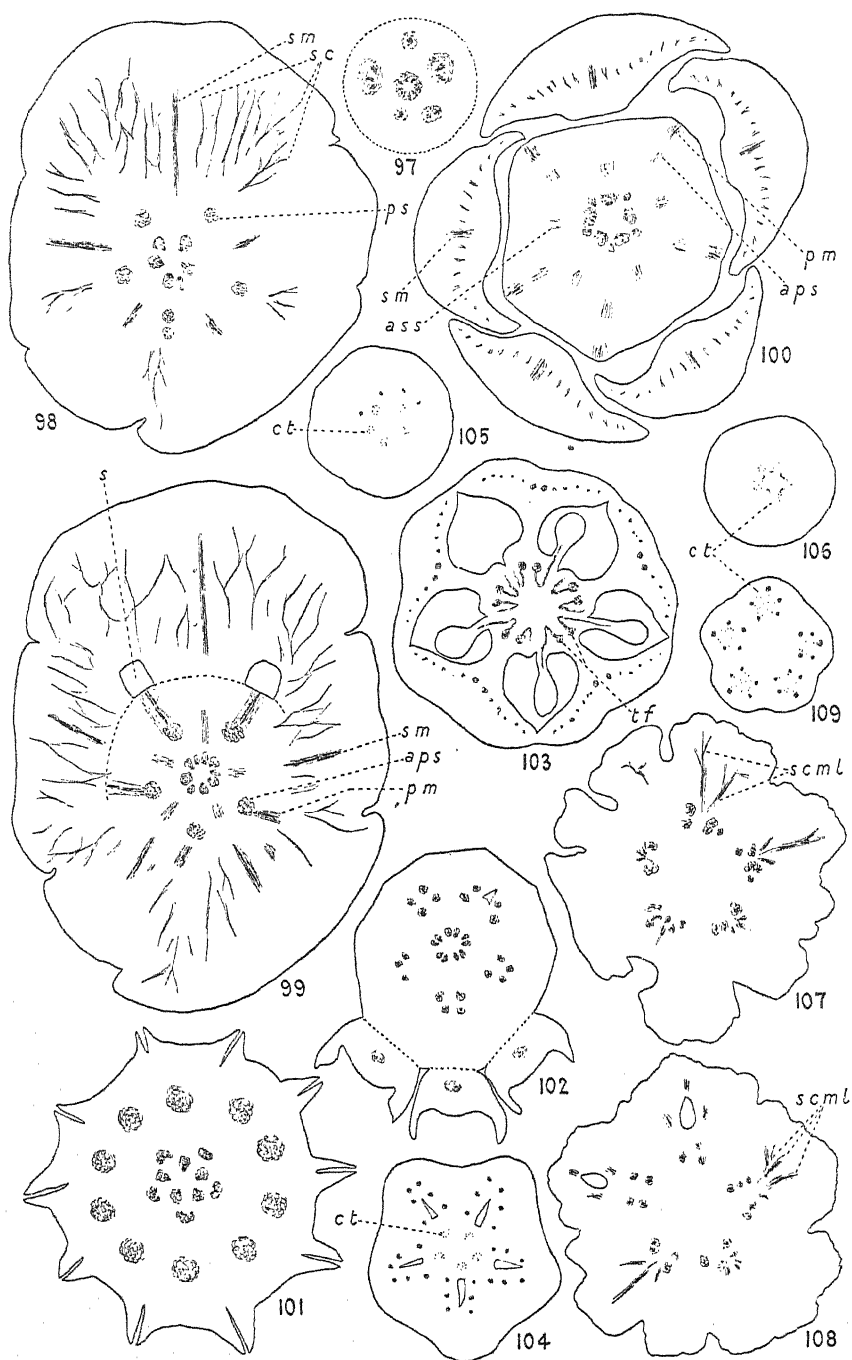
their components. On the other set of radii the trunk cords similarly show stages in the process of dissociation into sepal commissural marginals, petal midrib, and antepetalous stamen bundle. In the centre the residual vascular ring for the gynoecium. 90. The same after exertion of the perianth. At the periphery the stamen ring. In the centre the vascular bundles for the gynoecium already organized into an outer set of five for the sterile carpels in line with the petals and an inner set of five for the fertile carpels in line with the sepals. 91–3. Ovary base showing transition from a circular to a 5-angled outline, stages in the development of the loculi, and branching of the sterile carpel midribs. 94. Middle region of the ovary showing branches from the placental strands for the ovules. 95. Base of the style column which is solid owing to the persistence of the central parenchyma. On the radii of the petals and superposed upon the loculi five canals filled with conducting tissue. 96. Apex of the style column showing the five canals seen in 95 opening into a central space due to the disappearance of the central parenchyma.

dissociation does not take place until the cords are well free of the central cylinder, the successive stages in the process are exhibited with diagrammatic clearness.

In *Peganum Harmala* L. (Figs. 110–118), which has typically fifteen stamens, two standing in front of each petal, the organization of the bundles for the floral whorls also follows an unusual course. After the emergence from the central cylinder of the bundles for the five sepal midribs the residual vascular tissue is seen in transverse section as five tangentially elongated masses (Fig. 110). The next bundles to be defined are those for the superposed whorl of stamens. These are furnished by detaching from each of these residual masses a small end portion, this process leaving as before five tangentially elongated but somewhat smaller masses (Fig. 111). A large portion of each of these residual units shortly turns outwards, giving rise to a pair of sepal commissural marginals, the petal midrib and the two bundles for the antepetalous pair of stamens (Fig. 112). The remaining elements of each unit turn inwards to become organized later into the bundles for the two carpel whorls. The development of *two* stamens on each petal sector serves to illustrate a principle which has been observed in the course of the present series of investigations to underlie various, possibly all, cases of deduplication resulting in the appearance of *whole* members and to distinguish this process from the form of splitting which produces *half* members. Here, of the large above-mentioned vascular complex situated in each petal sector the central portion, which yields the sepal marginals and petal midrib and extends outwards in advance of the lateral portions, has a wide front (as seen in transverse section). Consequently the two lateral portions left behind lie some distance apart (see Fig. 112). We meet with conditions bringing about a like result in the geraniaceous genus *Sarcocaulon*, which also has a pentamerous flower with fifteen stamens, two standing in front of each petal. In this latter type the sepal marginals, petal midrib, and twin stamen bundles are organized successively from a single vascular complex. The two lateral portions of this complex destined for the two stamens are originally situated near enough together to come into contact. But this union is temporary. The sterile carpel midribs emerge almost immediately from the central cylinder and turn out horizontally. Their way to the periphery is blocked at first by these large stamen cords. Bipartition of the cords with resulting duplication of the stamens provides a way out (see 9, p. 102, Figs. 91–5, and p. 117). In this type as in *Peganum*, the twin bundles thus formed are derived from elements which already lie outside the central cylinder. Up till now the process of organization will presumably have been proceeding on normal lines and in both *Peganum* and *Sarcocaulon* will conceivably already have advanced to a point when these bundles make their appearance, at which alteration in the form of the resulting

structure cannot be effected. If this were the case the coming into being of *two* centres of further development would simply result in the appearance of two *whole* stamens, i.e. in deduplication. In the Fumarioideae, on the other hand, we have exemplified the type of splitting which results in *half* stamens. Here the bundles for the two lateral stamens are carried out conjoined with those of the two lateral petals while those for the two median members of the androecium turn out independently. At the moment at which the bundles for these latter members are due to turn outwards from the central cylinder the direct route is blocked by a continuous arc of the vascular elements which give rise to the outgoing trunk cord consisting of the conjoined midrib bundles of a median sepal and the superposed petal, flanked on the two sides by a closely packed group of elements, these two groups becoming consolidated later into one median carpel midrib. Emergence of a bundle to supply a median stamen from any point on this arc would appear to be precluded. The only alternative is for the elements concerned to make their way out round the ends of each arc. If the symmetry of the scheme of construction is to be preserved this entails the emergence of twin strands, one to the right and one to the left, in place of a single median bundle, both on the posterior and on the anterior side of the flower in order to supply the members of the androecium standing in the median plane. But this division in half and the separation of the vascular elements involved into two groups occurs at a much earlier moment here than is the case in *Peganum* and *Sarcocaulon*, and while these elements still form part of the unorganized central ring (see 6, p. 184, Figs. 44-7). Halving of the formative elements at this stage results in the formation of half stamens, stamens with filaments of about half the normal width and bearing half anthers. Nor is this a solitary instance of this type of splitting. A similar result attending halving of the group of the formative vascular elements set apart in each petal sector for supplying the antepetalous stamens is to be found in the Malvaceae, where also the antepetalous filaments bear half anthers.<sup>1</sup> In this family the division process often takes place several times, once at an early stage, as in the two types above described, and repeatedly later. After the emergence from the central cylinder of the bundles for the sepal midrib a large complex still forming part of the residual cylinder becomes defined on each petal radius. This complex supplies the sepal commissural marginals, petal midribs, and the antepetalous stamen bundles. The stamen formative elements of the petal-stamen component of this complex are from the outset divided into two groups separated by the wide arc of the petal component. This halving at this stage should, on the present interpretation, lead to the production of half stamens. That it does, in fact, do so is shown by the half anthers. Repeated splitting at a much later moment

<sup>1</sup> The antesealous filaments do not develop anthers.



FIGS. 97-109. 97-106. *Zygophyllum sessilifolium* L. 97. Flower base after the vascular elements for the sepals, petals, and antepetalous stamens have left the central cylinder irregularly combined in

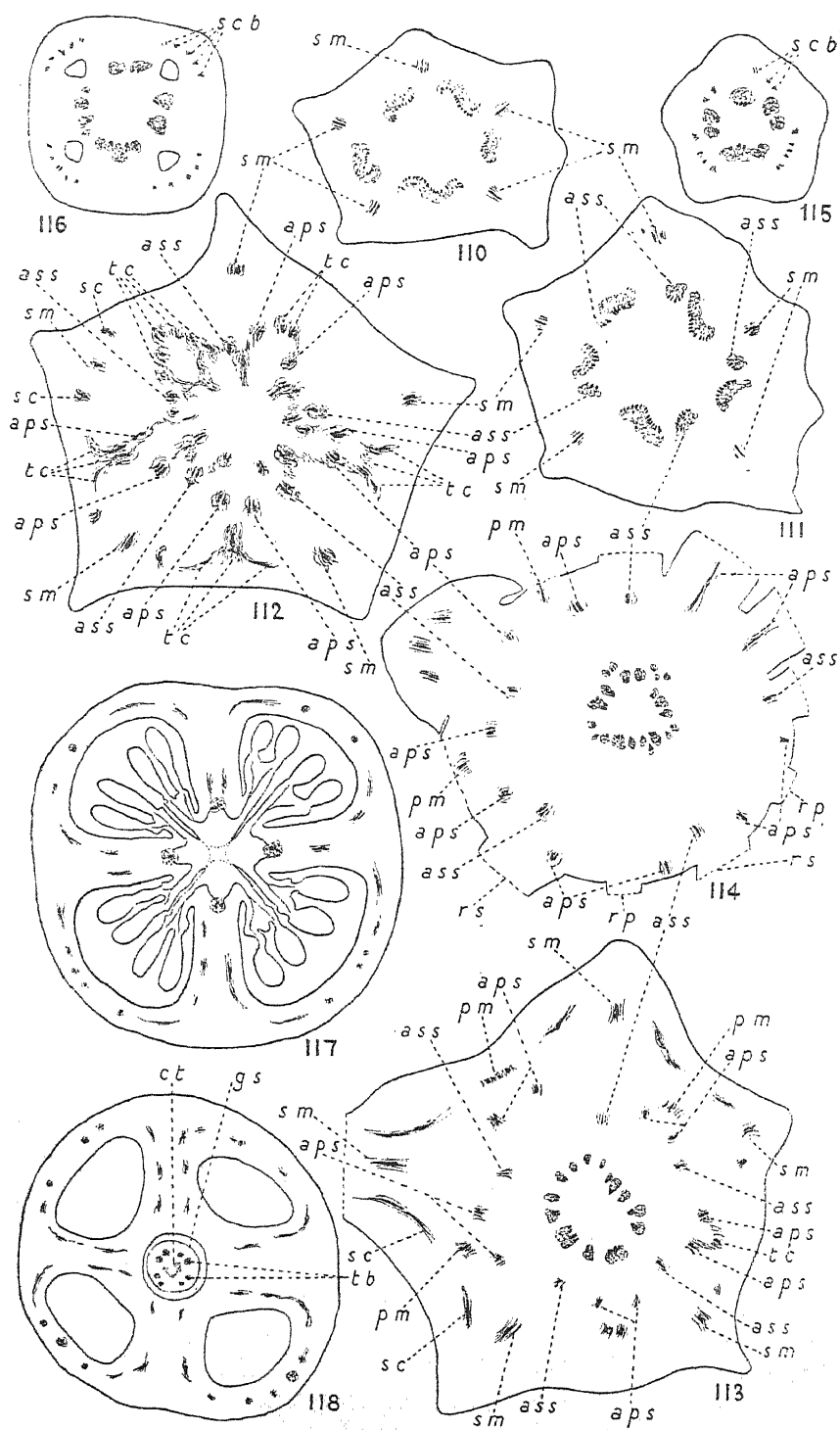


when they no longer form part of the central cylinder of the products of the original halving should, on the same view, simply produce an increased number of half stamens, which is the case.<sup>1</sup> In the gynoecium of *Peganum* the bundles for the sterile carpel midribs branch as they leave the central cylinder (Fig. 114), hence a group of strands instead of a single bundle enters the base of each sterile member (Figs. 115, 116). (A similar condition, as noted above (p. 662), and as shown in Fig. 35, is to be found in the rutaceous genus *Toddalia*.) The specimen of *Peganum* here figured was exceptional in having a tetramerous gynoecium (Figs. 115–18) in an otherwise pentamerous flower. Reference to Fig. 114 shows, however, that bundles for the sterile carpel whorl turned out from the central cylinder on five radii, but evidently in one case the issuing strand was too weak. No corresponding carpel was developed, this strand eventually forming part of the venation system of the neighbouring carpel (Figs. 115, 116).

In *Guaiacum officinale* L. (Figs. 119–25) the carpel whorls, as in the rutaceous genus *Ptelea*, though sometimes trimerous, are most frequently dimerous. In the allied form *G. sanctum* L. they are quite commonly pentamerous, though here also the number of carpels to the whorl may range down to two, as in *officinale*. In flowers with a pentamerous gynoecium the sterile carpels and loculi, as commonly in both Rutaceae and other Zygophyllaceae, stand in line with the petals. In such flowers the vascular

the form of five cords of very unequal size, two representing individual sepal midrib bundles, the other three a sepal midrib conjoined with the neighbouring petal midrib on the one, or on both sides. 98, 99. The same after the break-up of the large cords seen in 97 into their components = those proper to the sepal and the petal radii respectively. The latter, as in *Z. Faba*, yield commissural marginals for the sepals, petal midribs, and the bundles for the antepetalous stamens. In 98 the petal stamen cords have become detached from the sepal marginals which have given rise to a network. In 99 the petal stamen cords themselves are in process of becoming dissociated into their components (petal midrib and antepetalous stamen bundle). The posterior sepal is partly exerted. 100. The same after exertion of all the sepals. The bundles for the petals, which have been removed, are seen at the angles of the central pentagon. Nearer the centre the ring of bundles for the two stamen whorls, those for the antepetalous whorl having turned out independently from the central cylinder, those for the antepetalous being now all detached from the petal midribs. Differentiation of the bundles for the carpel whorls is in process of taking place. 101. The flower above the level of exertion of calyx and corolla. At the periphery the ring of stamen bundles. In the centre the bundles, now distinct, for the two carpel whorls. 102. Ovary stipe together with three stamens not yet exerted. Towards the periphery the branch systems of the sterile carpels and one loculus. In the centre on the alternate radii the twin-placental strands of the fertile carpels. 103. The ovule-bearing region of the ovary. The sterile carpel midribs which have given rise to a copious branch system are no longer traceable. In the centre the five pairs of placental bundles from which strands pass to the funicles. 104. The ovary just below the level at which the loculi close. The fertile carpel bundles have come to an end. In line with, and to the inside of each loculus an area of conducting tissue. 105. Lower region of the style column in which the sterile carpel systems, except for a few strands, have also come to an end. In the centre the five canals filled with conducting tissue. 106. Upper region of the same after the central parenchyma has come to an end giving rise to a space into which the canals open. 107–9. *Tribulus terrestris* L. 107, 108. The ovary before, and after, the appearance of loculi showing stages in the emergence and branching of the sterile carpel midribs. The residual strands for the fertile carpels fail to become consolidated into single cords before becoming differentiated into the placental strands. 109. Base of the short style column showing the persistent sterile carpel midribs and smaller strands around the large areas of conducting tissue.

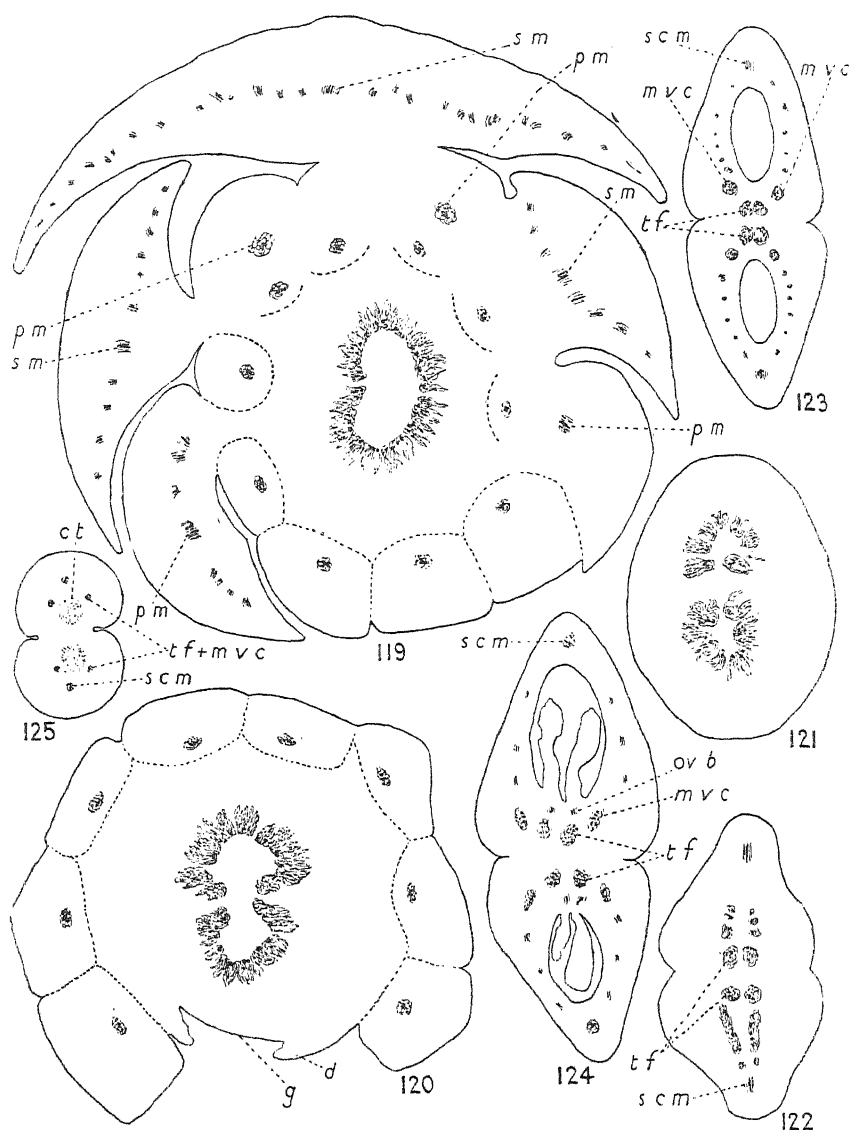
<sup>1</sup> It is proposed to deal in further detail with this point in a later communication.



scheme in the ovary will presumably show the ordinary ground-plan (I have not unfortunately been able to obtain specimens of such completely isomeric flowers for investigation). In flowers with an oligomeric gynoecium some modification of the full vascular scheme must obviously take place. Sections of a flower with a dimerous ovary show that after the emergence from the central cylinder of the bundles destined for the perianth and androecium the residual vascular elements form a complete cylinder (Fig. 119). As the stamens become exerted, leaving the gynophore exposed, the residual cylinder becomes divided into two (Figs. 120, 121). At the same time the bundle terminating each arm of each of the (in cross section) horseshoe-shaped vascular systems thus formed takes up a more internal position. This shifting has the effect of narrowing the distance between the free ends of each individual 'horseshoe' and of leaving a wide belt of parenchyma between the two horseshoes. At the level of transition from the gynophore to the ovary base the midribs of the two sterile carpels turn outwards from the midpoint of each horseshoe, and the bundles originally situated at the end of each arm of each horseshoe become organized into the twin bundles for each of two fertile carpels, the pair for one carpel being constituted of one bundle from the one horseshoe and one from the other. At the same time an external furrow makes its appearance on each side of the ovary in a plane at right angles to that in which lie the two sterile carpel midribs (Fig. 122). These furrows are the result of the partial splitting from without inwards of the two fertile carpels. The wide distance apart of the twin bundles of each fertile member which, as indicated in the preceding part of this account, is probably causally associated with the radial splitting of this member, results in the appearance of false pairs of bundles, each of the two bundles of one carpel standing nearer to the adjacent corresponding bundle of the other carpel than do the twin bundles belonging to one of the carpels to each other.

The gynoecium of *Guaiacum* thus occupies a position intermediate between that of the two rutaceous genera *Thamnosma* and *Ptelea*, in which it is also composed, typically, of two dimerous whorls. In *Thamnosma*

FIGS. 110-18. *Peganum Harmala* L. From a flower with a tetramerous gynoecium. 110. Flower base at the level at which the five sepal midribs turn out from the central cylinder leaving behind five large masses of vascular elements on the alternate radii. 111. The same after a portion of each of the residual vascular masses seen in 110 has become detached from one end to furnish the bundle for the antepetalous stamen on the adjacent radius. 112, 113. The same showing further stages in the break-up of the residual masses seen in 111. The bundles for the single antepetalous stamens can be identified on the one set of radii, and on the other set those for the sepal commissural marginals, petal midribs, and pairs of antepetalous stamens. The remaining portions of each vascular mass are turning inwards to become organized later into the bundles for the two carpel whorls. 114. The same after removal of all members of the perianth except one sepal (seen above on the left). On either side of the unexserted sepal a petal midrib bundle which has not yet wholly passed out from the axis. In an outer ring the bundles for the fifteen stamens. In the centre the residual vascular ring from which groups of strands which supply the sterile carpels are beginning to emerge. 115. The gynophore. 116. Ovary base. 117. Ovule-bearing region of the ovary. 118. Upper region of the ovary with 'gynobasic' style column.



FIGS. 119-25. *Guaiacum officinale* L. From a flower with a dimerous gynoecium. 119. Flower base after exertion of two of the five sepals and one of the five petals. The ring of ten stamens is becoming defined. In the centre the vascular ring which serves the gynoecium about to become divided in half transversely. 120. The same after exertion of the remaining perianth members and two of the stamens. The thin layer of disc tissue surrounding the base of the gynophore has come to an end on the radius of one of the exerted stamens exposing the surface of the gynophore. 121. The gynophore now wholly free. 122. Ovary base. The bundles for the midribs of the two median valve carpels have turned outwards and are seen cut obliquely, those for the secondary veins, retaining their position, are cut transversely. Right and left the twin bundles for the two lateral fertile carpels, already showing slight radial splitting. 123. The same after the appearance of the loculi. The marginal veins of the valve carpels have now become prominent. 124. The ovule-bearing region of the ovary. 125. Base of the style column. In the median plane the two sterile carpal midribs. On each side of each midrib a strand formed by the fusion of a marginal vein with one of the twin bundles of the lateral fertile carpal on that side. In line with the two midribs two areas of conducting tissue.

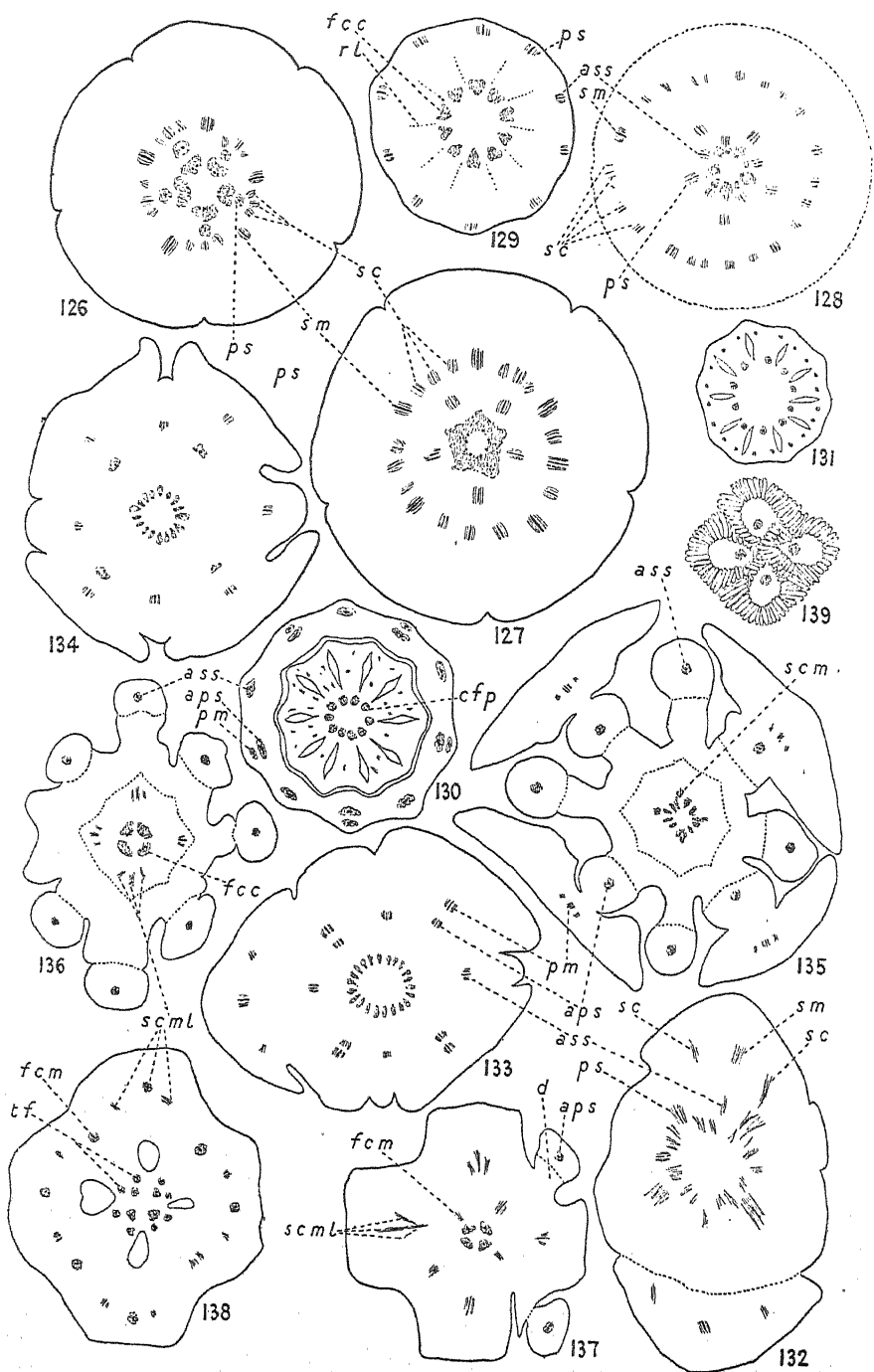
both kinds of carpels contribute, as usual, to the ovary wall; the pith comes early to an end, the ovary then becoming unilocular. As radial splitting of the fertile members becomes complete the gynoecium below the style column becomes wholly, though spuriously, apocarpous. In *Guaiacum* the ovary wall is also formed of all the carpels. The pith persists; radial splitting of the fertile members is incomplete; hence the gynoecium remains bilocular and syncarpous throughout. In *Ptelea* the fertile carpels are completely enclosed by the sterile members, hence there is no radial splitting, the ovary remaining syncarpous throughout and giving rise to an indehiscent fruit. As in *Thamnosma*, however, the pith ceases early and the ovary becomes unilocular.

*Augea capensis* Thunb. (Figs. 126-31). In the development of the perianth and stamen bundles *Augea* resembles the majority of rutaceous types, the marginal veins of the sepals being commissural in origin (Figs. 126-8), the bundles of the antesealous stamens arising independently (Fig. 129), those of the antepetalous whorl turning out conjoined with the petal midribs (Figs. 127-9). The gynoecium, however, is exceptional in that the number of loculi, and hence of each kind of carpel, is twice that of the members in each perianth whorl. All the members of each carpel whorl constitute a single ring. Midrib bundles are lacking in both sets of carpels. We may infer, however, since the loculi make their appearance on the radii alternating with both sets of perianth members, that these intermediate radii represent the mid-line of the sterile carpels and hence that in their arrangement they treat two preceding whorls as one, an arrangement resulting, no doubt, from the fact that petals and antesealous stamens are conjoined at their base into a continuous ring of tissue, the bundles of the antepetalous stamens not becoming disjoined from the petal midribs until much later (Fig. 130). In accordance with this sequence the cords of the fertile carpels are situated in line with the perianth members, branches from these cords extending outwards round the loculi into the wall of the ovary.

#### Stachyuraceae (Figs. 132-9).

The one genus included in this family, *Stachyurus* has an isomerous tetramerous flower with  $G\ 4+4$ .

*Stachyurus praecox* Sieb. and Zucc. This species affords an example of the exceptional case in which a dicotyledon flower with the full number of whorls and isomerous throughout is diplostemonous. Here, as in most Rutaceae, the sepals have commissural marginal veins, the bundles for the antesealous stamens arise independently, those for the antepetalous members turn out conjoined with the petal midrib bundles. The obdiplostemonous condition which we might expect to prevail in these circumstances is not possible owing to the particular shape of the disc on which

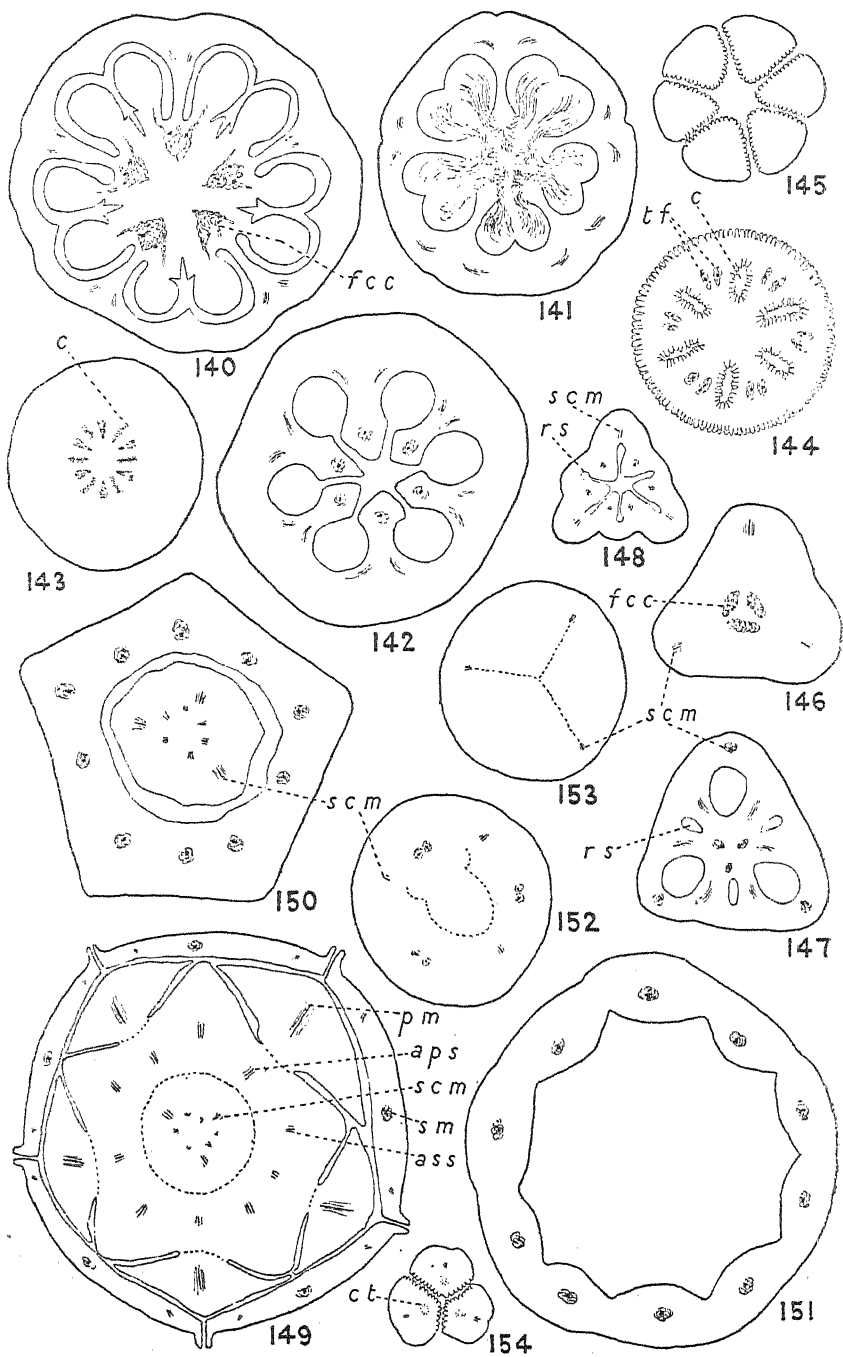


the stamens are seated, which carries the antesepalous members out beyond the antepetalous whorl which is further prevented from attaining a more outward position by the form and position of the petals with which they are still fused on their outer face.

Meliaceae (Figs. 140-5).

In the two genera examined, *Turraea* and *Melia*, the trunk cords which turn out on the petal radii give rise to sepal commissural veins and petal midribs, but the bundles for the antepetalous stamens, as well as those for the antesepalous members of the androecium, turn out independently from the central cylinder.  $G = n + n$ ,  $n$  varying from 4 to 20. Midrib bundles are no longer traceable either in the sterile or the fertile carpels. As the loculi make their appearance the ring of residual strands seen at a lower level become consolidated into a determinate number of bundles standing at first in line with the loculi. Before the ovule-bearing level is reached these strands become reorganized, the resulting placental cords being now disposed in line with the septa (fertile carpels). As the pith comes to an end the fertile carpels separate along their inner face and

FIGS. 126-39. 126-31. *Augaea capensis* Thunb. 126. Flower base at the level of origin of the sepal midrib bundles on the one set of radii and of the sepal commissural marginals and petal-stamen bundles on the alternate set. 127. The same after the petal-stamen bundles (seen cut transversely in 126) have turned out horizontally. In the centre the residual vascular elements, forming a continuous ring. 128. Central region of the same at the level at which the antesepalous stamen bundles turn out from the central ring. 129. The flower after exertion of the calyx. At the periphery a ring of ten bundles, the five for the antesepalous stamens alternating with the five trunk cords for the petal midribs and antepetalous stamen bundles. In the centre the residual vascular tissue for the gynoeceum. The position of the loculi which appear on the intermediate radii is indicated by the interrupted radial lines. 130. The same after the petal-stamen ring has become disjoined from the gynoeceum. The antepetalous stamen bundles are now detached from the petal midribs. In the gynoeceum the bundles for the fertile carpels seen in 129 have divided in two, each half fusing with the adjacent half of the neighbouring carpel bundle so that the resulting bundles come to stand in line with the loculi. 131. The ovary just before the loculi close. The main bundles of the fertile carpels have been reformed (by reversal of the process which brought about the transition from the condition seen in 129 to that in 130) and now alternate with the loculi as at first. 132-9. *Stachyurus praecox* Sieb. and Zucc. 132. Flower base. Back and front the two median sepals, with midrib and commissural marginals, immediately before exertion. In line with these sepals and just free of the central vascular ring the bundles for the two median stamens. In the lateral plane the bundles for the two lateral sepals not yet free of the central cylinder. In the diagonal planes the four trunk cords furnishing the sepal commissural marginals, petal midribs, and antepetalous stamen bundles. [Owing to the late development of the lateral sepals the commissural marginals for the median sepals become detached from these diagonal trunk cords before those for the lateral sepals.] 133. The same after exertion of the median and right lateral sepals and complete dissociation of the diagonal trunk cords into their components. 134. The same after exertion of the remaining sepal. 135. The flower just before exertion of the petals showing a diplostemonous arrangement as the result of the peculiar shape of the disc on which the stamens are seated. [The boundaries of the disc where it is not yet disjoined from androecium and gynoeceum are indicated by interrupted lines.] In the centre the bundles for the four sterile carpel midribs are about to issue from the central cylinder in the orthogonal planes (i.e. in line with the sepals). 136. The same after exertion of the petals and one of the antesepalous stamens. The sterile carpel midribs have given off a pair of lateral branches. On the alternate radii the bundles for the four fertile carpels have become consolidated. 137. The ovary to which are still attached remnants of the disc carrying two of the inner whorl of stamens which are not yet exerted. In the diagonal planes the fertile carpel midrib bundles which have turned out from the central cylinder leaving behind the placental strands. 138. The same after the appearance of the loculi. 139. The four stigmas which are centred over the loculi.





the ovary becomes unilocular. In *Melia Azedarach* L. the placental surfaces again become continuous as the ovary passes into the style filament, a transverse section of this region showing the persistent placental cords alternating with a corresponding number of stylar canals. In *Turraea mombassana* Hiern, the placental surfaces, after becoming free, remain separate, the single loculus passing up into one central stylar canal which is seen in transverse section of a pentamerous gynoecium as a five-armed area of conducting tissue.

#### Cneoraceae (Figs. 146-8).

*Cneorum tricocon* L., G 3 + 3. The flower is typically isomerous and trimerous throughout; only the antesealous stamen whorl is developed, hence the sterile carpels and loculi naturally develop in line with the petals. Radial splitting of the fertile carpels is initiated as an interstitial slit. With the disappearance of the pith the ovary becomes unilocular, the cavity appearing six-armed in transverse section as the interstitial slits extend to the centre. The ovary, however, remains syncarpous, extension of the slits to the exterior only taking place at the base of the style column.

#### Erythroxylaceae (Figs. 149-54).

*Erythroxylon Coca* Lam. In *Erythroxylon*, as in the majority of types already described, the bundles for the antesealous stamens turn out independently, those for the antepetalous members form part of the trunk cords which give rise to the sepal commissural marginals and petal midribs. Although here the filaments are united into a tube and there is no disc, the

FIGS. 140-54. 140, 141. *Turraea mombassana* Hiern. The ovary of a pentamerous flower. 140. From the multilocular, ovule-bearing region. 141. From the region above the ovules. The central parenchyma has come to an end causing the ovary to become unilocular, the single cavity being partly filled with the numerous hairs now clothing the placental surfaces. 142-5. *Melia Azedarach* L. From a hexamerous flower. 142. The unilocular ovary (for simplicity the ovules have been omitted). 143. Base of the style column. The vascular strands of each fertile carpel have become consolidated into a single bundle. On the alternate radii (hence in line with the loculi) the six stylar canals. The fertile carpels having now come into contact along their inner faces the central cavity seen in 142 has become obliterated. 144. Apex of the style column at the level of transition to the stigma. The fertile carpel bundles have divided in half preparatory to the separation of the six stigmatic lobes. 145. The six stigma lobes which are centred over the loculi. 146-8. *Cneorum tricocon* L. 146. Base of the ovary after the sterile carpel midrib bundles have turned out from the central cylinder. In the centre, on the alternate radii, the cords for the fertile carpels. 147. Middle region of the ovary. In the mid-line of each fertile carpel an interstitial radial split. 148. Upper region of the ovary now become unilocular owing to the disappearance of the central parenchyma. The interstitial radial slits seen in 147 have extended to the centre so that the single loculus is 6-rayed. 149-54. *Erythroxylon Coca* Lam. 149. Flower base showing the exerted sepals, the petals just before exertion, and the staminal ring defined, but not yet disjoined from the trimerous gynoecium. The bundles for the antesealous stamens stand slightly farther out than those for the antepetalous members (see explanation, p. 688). In the centre the midrib bundles for the three sterile carpels and the twin strands for the three fertile members. 150. The staminal ring and gynoecium now disjoined. 151. The staminal ring. 152. The ovary just before the appearance of the loculus (indicated by the interrupted line), of irregular shape owing to only one of the three fertile carpels being functional. 153. The same after the loculus has become closed. 154. The three stigma lobes which are centred over the loculi, each with sterile carpel midrib and an area of conducting tissue.

whole configuration is such as to produce at first a diplostemonous arrangement as in *Stachyurus*. Previous to exertion the petals are packed between the enclosing sepal edges to the outside and the staminal ring on the inside. Their shape causes an indentation in the ring on these radii, hence, while the antesealous stamens come to stand farther and farther from the centre, the antepetalous members are being prevented from taking up a position farther out, so that the flower at this level is diplostemonous. In the oligomerous gynoecium ( $G\ 3\ or\ 4 + 3\ or\ 4$ ) only one of the potentially fertile carpels is usually functional. As the loculi become closed the fertile carpel bundles are no longer traceable, the sterile carpel midribs alone passing up into the separate styles.

#### SUMMARY AND CONCLUSIONS.

Except where individual families are cited the following statements, in so far as they are applicable, hold good for the several families Rutaceae, Zygophyllaceae, Meliaceae, Cneoraceae, Erythroxylaceae, and Stachyuraceae:

1. The sepals are usually furnished with commissural marginal veins arising in the typical manner as components of trunk cords turning out from the central cylinder on the petal radii. In a minority of species the marginal veins either arise through true lateral branching of the midrib or are lacking, the midrib remaining unbranched or branching only near the apex.

2. A study of the floral anatomy entirely confirms the view previously put forward that the fundamental principle underlying the radial relations of successive floral whorls is not primarily the alternation of the members of successive whorls, though this may result, but the alternation of the sets of vascular bundles turning out from the central cylinder to supply these members. When midrib bundles for successive whorls turn out independently the members of successive whorls as well as their midrib bundles regularly alternate. When the bundles for superposed members turn out conjoined as trunk cords the alternation of sets of outgoing bundles continues unchanged, but the alternation of whorls is broken at this point. The most commonly occurring example of such a break in the regular scheme of alternation is seen in those completely isomerous types in which petal midrib and antepetalous stamen bundle turn out from the central cylinder conjoined into a single trunk cord with resulting obdiplostemony and antepetalous loculi. These differences in the manner of origin of the vascular bundles of successive whorls enables us to understand how it comes about that diplostemony and obdiplostemony, antesealous and antepetalous loculi, may occur among related forms having the same floral ground-plan, and why the suppression of the inner stamen whorl, even

when not epipetalous, is able to occur without affecting the radial position of the succeeding whorls.

3. The normal occurrence in many types of one set of trunk cords, from which are derived the midrib bundles for two successive whorls of members situated on the corresponding radii, throws light on the cause of the 'loss' when a whorl is suppressed. Suppression in the above circumstances may not imply complete failure to initiate any stage of the development of the missing members, but the lesser deviation from normal of the failure to detach a component from an existing bundle.

4. The facts observed afford further evidence in support of the view previously expressed that in the Dicotyledon flower which is isomerous throughout diplostemony, in consequence of cumulative 'congestion' effects, is comparatively rare, only being met with in those forms where some special structural configuration either affords the necessary 'relief' from this state of congestion, as when a gynophore is developed, whereby the level at which radial extension of the ovary occurs is raised above that at which the 'blocking' effect due to encirclement by the earlier outer whorls has diminished. Or, introduces dissimilar conditions on the two sets of radii, as when the disc carrying the stamen filaments is of such a shape that the distance of its free border from the axis centre is greater on the sepal than on the petal radii, so that the antesepalous stamens are carried farther out than the antepetalous members.

5. The present interpretation of the cause of genuine obdiplostemony in association with antepetalous loculi in the Dicotyledon affords an explanation of the curious and hitherto unexplained circumstance of the complete absence of these features among Monocotyledons. For in Monocotyledons the conditions in which these relations are found characteristically in Dicotyledons are never present.

6. The original relative position as regards distance from the axis centre of the vascular bundles for the two stamen whorls before exertion of these members may be modified after exertion by secondary causes such as dense packing or the union of the filaments at their base into a tube.

7. The gynoecium in each of the six above-mentioned families, as in all other syncarpous types so far investigated, is found to consist of two carpel whorls, the outer sterile, the inner fertile. The ground-plan in all types is expressed as  $Gn+n$  but  $n$  may vary from 1 to 20.

8. The vascular system of the gynoecium shows very generally a marked degree of degeneration. In the majority of genera the fertile carpel bundle corresponding in position with the sterile carpel midrib is lacking, while in many the sterile carpel midrib has also disappeared completely, or is but feebly developed and soon ceases to be traceable. This general condition appears to be associated with the development of an abundance of oil-secreting cavities (most Rutaceae) or of individual cells

rich in oil or in mucilage (*Augea*). In the Zygophyllaceae, in which oil glands are lacking, the sterile carpel midribs are comparatively well developed.

9. The pseudo-apocarpous condition characteristic of most rutaceous genera arises from radial splitting of the fertile carpel members and may be compared with that seen in *Sterculia*. The split may extend from the outer surface inwards to the ventral face; or, it may appear first as an interstitial rift in the mid-line of the fertile carpels, which later extends in one or both directions; or, again, where the pith comes early to an end, it may begin at the inner face and extend outwards.

10. The relation in respect of the vascular scheme of the wholly syncarpous to the spuriously apocarpous types in the six above-mentioned families may be compared with that existing between those genera in the Liliaceae, *Fritillaria*, *Tulipa*, *Lilium*, which have a fertile carpel main bundle corresponding in position with the midrib bundle of the sterile carpel, and are without 'septal' glands, and those constituting the rest of the family which, conversely, have no such bundle and possess 'septal' glands.

11. The level at which transition from the syncarpous to the spuriously apocarpous condition becomes complete depends, as a rule, upon the level at which the pith tissue comes to an end. In the Zygophyllaceae and in the few Rutaceae in which the pith persists to the apex the ovary remains syncarpous.

12. The individual ovaries, which result from radial splitting of the fertile carpels, are not monocarpellary as hitherto conceived, but consist, as their method of origin indicates, of a whole sterile carpel together with half the adjacent fertile carpel on each side, i.e. of  $\frac{1}{2} + \frac{1}{2}$  carpels. In a type where, through reduction, only one sterile and one fertile carpel are developed, as in *Cneoridium* (Rutaceae), in which consequently there is only one ovary present, a radial split in the mid-line of the fertile member results in a similar configuration.

13. The two-carpelled ovary of *Cneoridium* presents a resemblance in a transverse section taken below the level of origin of the 'gynobasic' style filament to the two-carpelled ovary of the Leguminosae, in which radial splitting of the fertile member also takes place for an extremely short distance at the ovary base. But in this latter family, where the style springs from the summit of a greatly elongated ovary, almost the whole length of the ovule-bearing region intervenes between the opening so formed connecting the loculus with the exterior and the base of the style, whereas in *Cneoridium* the 'gynobasic' style arises immediately above the level of closure of the passage.

14. The individual style filaments when free, and the unit components of the style column when fused, stand in line with the sterile carpels in all

the above-mentioned six families. When partially or completely connate each, probably, always represents the prolongation of  $\frac{1}{2}$  +  $\frac{1}{2}$  carpels. In male flowers in which the ovary is syncarpous but the styles are distinct only the sterile carpels are prolonged to form the filaments. This is very probably the case also in the hermaphrodite flowers of *Erythroxylon Coca* in which the styles are free and receive only the sterile carpel midrib bundles.

15. The separate stigmas or unit stigmatic lobes are centred over the sterile carpels and hence over the loculi.

16. In those declinous types in which the gynoeceium of the female flowers remains syncarpous throughout while the male flowers develop a sterile gynoeceium open at the top and with separate style filaments, these features of the male gynoeceium are due to the suppression of the inner, fertile carpel whorl. Consequently placentae, placental bundles and ovules are absent. Furthermore, as the pith comes to an end the small slit-like loculi become continuous, giving rise to a single cavity, open above to the exterior for the short distance before the sterile carpels, unable to cohere with each other, separate to terminate in distinct styles, as in *Phellodendron*, *Evodia*, and probably *Vepris*. This relation between the ovaries of the two sexes is precisely similar to that exhibited in still more striking fashion in species of *Paepalanthus* (Eriocaulaceae) in which the ovary of the male flower is three-styled and that of the female flower six-styled.

17. In those diclinous types in which the gynoeceium of both female and male flowers becomes spuriously apocarpous and the styles remain distinct, as in *Zanthoxylum*, the sterility of the male flowers is similarly due to the suppression of the fertile carpels, the ovaries being without loculus, placentae, placental bundles, and ovules, each being composed of only a single carpel.

18. In the diclinous flowers of *Ptelea*, in which the ovary wall is formed entirely from the sterile carpels, the inner whorl of fertile carpels is present in the male as well as the female flowers, but in the male flowers they do not reach full development, the ovules being aborted.

19. In the female flowers of *Ptelea* a definite bundle (starch) sheath surrounds the central vascular cylinder between the levels at which the bundles for the sterile carpels turn outwards and those for the fertile carpels are organized, a feature difficult to reconcile with the traditional view that only one whorl of carpels is present, but quite compatible with the interpretation that  $G = 2 + 2$ .

20. The inability of the sterile carpels, when present alone, to remain conjoined, which is characteristic of the style region in the male flowers of *Phellodendron*, *Evodia*, and *Vepris*, is seen in a more extreme form in the variety *digitata* of *Citrus medica* subspecies *Limonum*, in which it is inferred that the fertile carpels are similarly suppressed since loculi,

placentae, and ovules are lacking. Here the sterile carpels appear as so many finger-like structures, which are separate almost from the base.

21. The appearance that the unit stigmas in some species of *Boronia* (*B. megastigma*, *B. Purdieana*) (Rutaceae) are commissural and centred in line with the sepals in contrast with other species of *Boronia* and with most other Rutaceae, in which they are centred over the petals, is illusory. The unit stigmas in *megastigma* and similar species correspond, not with the four arms of the stigmatic cross, but with the four alternate V-shaped sectors which are centred midway between the arms. Each of the four arms represents the termination of one whole fertile carpel, which is split radially in two; each of the V-shaped sectors, i.e. each unit stigma, consists of one whole carpel flanked on each side by half of the neighbouring split fertile carpel.

The accompanying drawings were made by Miss D. F. M. Pertz, to whom I here tender my very grateful thanks.

I am also greatly indebted for material to the Director of the Royal Botanic Gardens, Kew, to the Directors of the Botanic Gardens at Cambridge, Calcutta, and Trinidad, to Professor F. E. Clements, and Mrs. M. R. Levyns.

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# A Chromosome Survey of Aconitum. I.

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With twenty-seven Figures in the Text.

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## INTRODUCTION.

*ACONITUM* is a large genus with its maximum of diversity in the mountainous borderlands of Central Asia. It falls into three groups according to the nature of the underground portion of the plant. The first group, corresponding to the section *Gymnaconitum* of Stapf, contains only the species *A. gymnandrum*, Stapf, an annual. It will not be considered

here. The second group, corresponding to the section *Lycototum* of De Candolle, has a perennial rhizome from which the flower-stems arise each year. The third group, comprising the large section *Eu-Aconitum* of C. A. Meyer and the small section *Anthora* of De Candolle, has tubers. The individual tubers are monocarpic; each puts up one flowering stem and then gives rise to one or more tubers from lateral buds.

The section *Lycototum* extends from Europe right across northern Asia to Japan and Sakhalin and south to the western Himalayas, with one species in Morocco and one in eastern N. America.<sup>1</sup> The species when in flower are as a rule easy to recognize on account of the tall helmet. The colour of the flowers is most generally a pale waxy yellow or a dingy purple.

The small section *Anthora* forms a natural group of somewhat more restricted distribution. It differs from the rest of the genus in having persistent sepals. The colour of the flowers is almost always yellow. The section is mainly represented in Northern Asia and Central Asia, with outlying stations in the Caucasus, in eastern and southern Central Europe and the Pyrenees, and in Western China. It does not occur in America.

The remainder of the genus forms the section *Eu-Aconitum*. The roots are tuberous, the sepals deciduous. The flowers may be any shade of blue or purple, occasionally yellowish-white. The helmet is variable in shape and height, but rarely equals the elongation found in the section *Lycototum*. Species of *Eu-Aconitum* cover the whole of Europe and temperate Asia, and range to North America.

In the last half-century a large number of Asiatic and American species have been described. In China and Inner Asia the genus is represented by a long list of species ranging in habit from *A. Hemsleyanum*, a scandent form growing 9 ft. high in bushes, to *A. naviculare* and *A. Hookeri*, growing a few inches high with at the most three or four flowers on a stem. The commonest Far Eastern aconites in cultivation are *A. Wilsoni* Stapf, and *A. chinense* Sieb., which have large flowers and large thick leaves, the lower ones at least with broad segments, and flower quite a month later than the blue-flowered European forms.

There is no monograph more recent than that of Stapf (13) on the Indian and Gayer (4) on the European species, and the newer species from China and Inner Asia await detailed classification.

The present paper deals with the somatic chromosome complements of

- (1) the section *Lycototum*,
- (2) the section *Anthora*,
- (3) a small number of Asiatic *Eu-Aconiti* of various affinities,

<sup>1</sup> The affinities of *A. reclinatum* are not beyond question, but Gray (6) definitely referred it to the section *Lycototum*.



(4) the sub-divisions of the European *Eu-Aconiti*, and the hybrids between them.

Previous studies have been made by Langlet (9, 10), Lewitsky (11), Darlington (3), and Afify (1). We are indebted to Darlington for certain of the illustrations in the text (Figs. 16, 21, and 22).

A key to the sections, a provisional arrangement of the Asiatic *Eu-Aconites* and some tabulated information about the European species will be found in Appendix I.

#### MATERIAL.

All the available particulars as to the origin of the material will be found in Appendix 3.

#### METHODS.

The fixative used for root-tips was 2 BE (8). Sections were cut at  $26\mu$  and stained by Newton's gentian violet method (8).

Drawings were made with a 1.5 Zeiss apochromatic objective and a  $\times 30$  compensating ocular, with the aid of an Abbé camera lucida, giving a magnification of roughly 6,000, and have been reduced in reproduction to the magnification shown.

#### *Section Lycoctonum.*

Five species have been examined, viz.:—

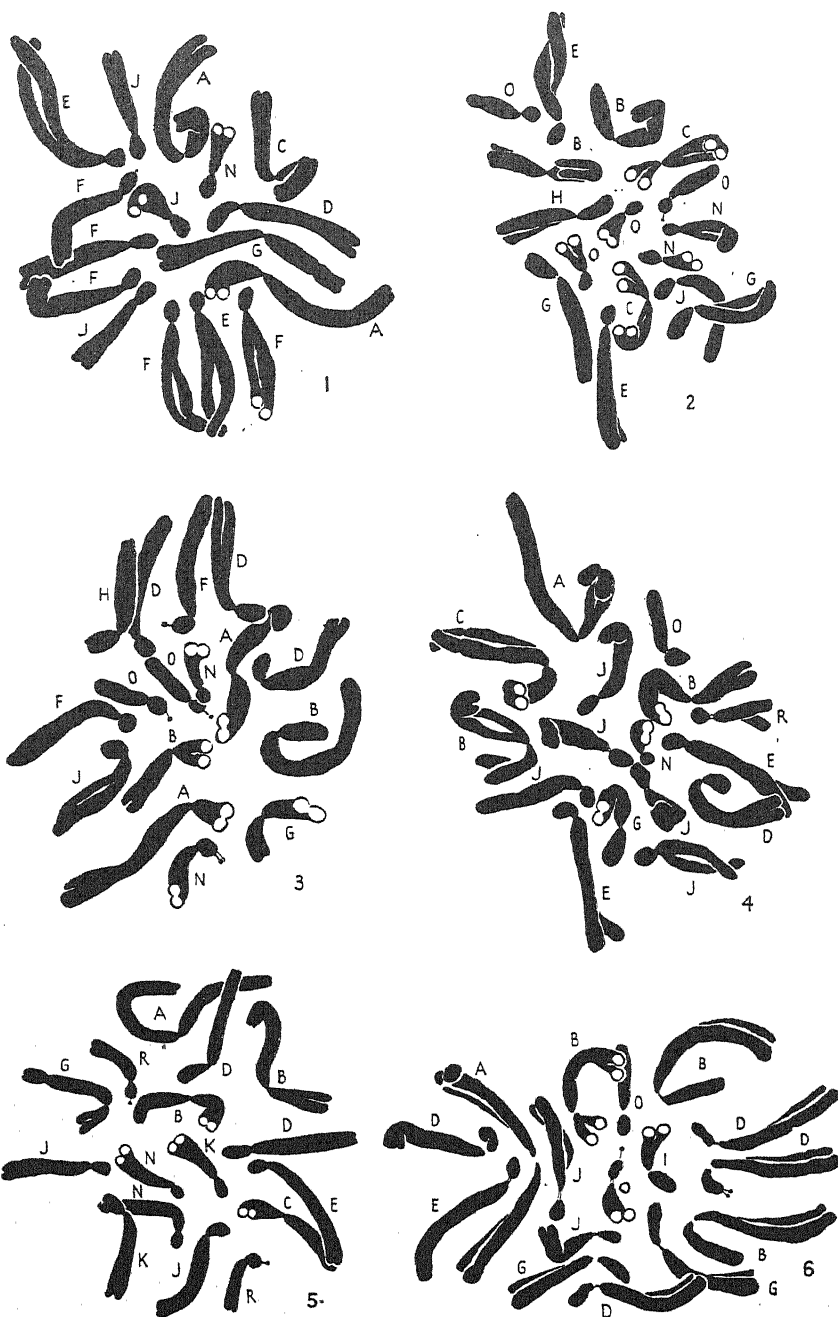
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|--|----------|----------------------------|
| 1. <i>A. vulparia</i> Rchb.                                    | 5 clones | } All diploid, $2n = 16$ . |
| 2. <i>A. orientale</i> Boiss.                                  | 3 clones |                            |
| 3. <i>A. Lycoctonum</i> L. (= <i>A. septentrionale</i> Koelle) |          |                            |
| 4. <i>A. luridum</i> Hook. fil. et Thom.                       |          |                            |
| 5. <i>A. thyriacum</i> Blocki.                                 |          |                            |

*A. vulparia* shows considerable variation in the type of inflorescence, dissection of leaf, vigour, &c. Some of the clones were coarse growers with dissected ranunculoid leaves, while one (21/33) was the Alpine type with moderate growth and broad leaf segments. *A. orientale*, from the Near East, differs from *A. vulparia* in its narrow elongated helmet. The remaining species, *A. Lycoctonum* L. from Sweden, *A. luridum* from Northern India, and *A. thyriacum* from Galicia, all have flowers of some shade of purple.

As shown above the chromosome number was constantly diploid,  $2n = 16$ . The appearance of the complement was characteristic (see p. 705).

#### *Section Anthora.*

It is a question whether *A. Anthora* can be divided into good sub-species or varieties. It varies to some extent in stature, branching, width



FIGS. 1-6. Somatic metaphases in aconites of the section *Lycocotum*. All diploid,  $2n = 16$ . Fig. 1, *A. luridum*, with the greatest total chromosome length observed in any diploid species. Fig. 2, *A. Lycocotum* L., with the lowest total length in the section. Figs. 3, 5 and 6, different clones of *A. vulparia* (28/30, 21/33, 17/33). Fig. 4, *A. orientale*.  $\times 3,000$ .

of leaf segments and shape of hood, but is always readily recognizable and distinct from all other species.

Only two stocks have been obtained. These were tetraploid,  $2n = 32$  (Fig. 16). Langlet and Lewitsky also report tetraploid forms.

### Section *Eu-Aconitum*.

#### (a) *Middle and Far Eastern Eu-Aconites*.

The following have been examined :

##### *Dwarf group*<sup>1</sup>.

<i>A. heterophyllum</i> Wall.	$2n = 16$ (Fig. 7)
<i>A. Toppinii</i> Stapf	$2n = 16$

##### 'Chinese' or *paniculate group*.

<i>A. transectum</i> Diels	$2n = 16$ (Fig. 9)
<i>A. Forrestii</i> Stapf	$2n = 16$ (Fig. 8)
<i>A. chinense</i> Sieb.	$2n = 32$ (Fig. 19)
<i>A. ? spicatum</i> (Hay 414) <sup>2</sup>	$2n = 32$
<i>A. sp.</i> (Hay 409)	$2n = 32$
<i>A. palmatum</i> (or hybrid)	$2n = 45-47$
<i>A. ? palmatum</i> (Hay 12)	$2n = 48$
<i>A. Wilsoni</i> Stapf	$2n = 64$

##### *Scrambling group*<sup>3</sup>.

<i>A. ? Hemsleyanum</i> Pritzel (Kew)	$2n = 16$
" " " (Hay)	$2n = 16$
" " " (Stern)	$2n = 16$
<i>A. ? volubile</i> Pall.	$2n = 32$

Any detailed discussion of the Middle and Far Eastern *Eu-aconites* is out of the question until much more material has been obtained. Referring to the working classification, Appendix 1, it will be seen that three groups out of five are represented. In the dwarf group, the two forms examined are diploids. In the Chinese group, the leafy, paniculate type, there is a series of diploid, tetraploid, hexaploid, and octoploid species. In the scrambling group, of the four distinct clones examined, the three diploids evidently belong together, and the fourth, a tetraploid, is very different. Of the three diploids two are known to be Himalayan. Nothing is known of the origin of the other two clones, but the tetraploid on morphological grounds is probably *A. volubile* Pall., a Siberian plant.

Langlet reports one of the scrambling group, *A. volubile latisectum* (see Appendix 2) as diploid,<sup>4</sup> and also one of the Chinese group, *A. kamtschaticum* (= *A. maximum* Pall., see Hultén, 1928). He agrees with us in obtaining the octoploid number for *A. Wilsoni*.

<sup>1</sup> See Appendix I (ii) for classification adopted.

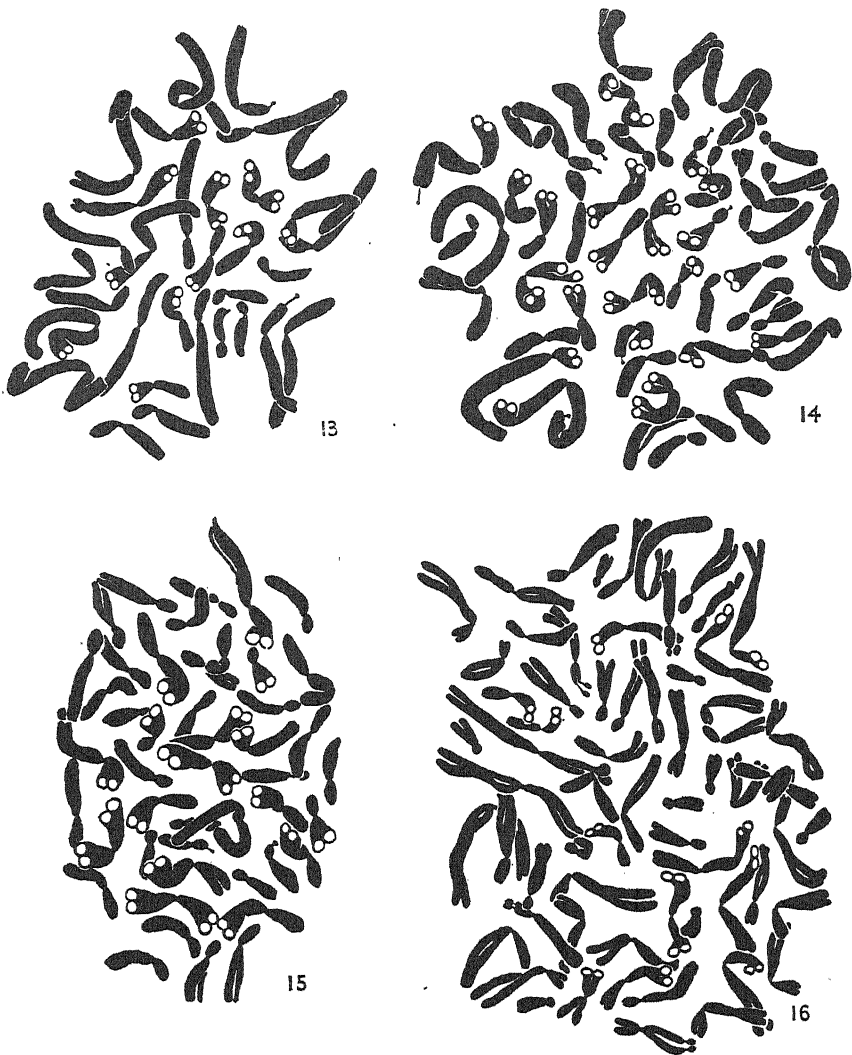
<sup>2</sup> Mr. Hay's plants were grown from seed collected in Nepal. They were sent here under number and have not all been named with certainty.

<sup>3</sup> The names in the scrambling group are in a state of great confusion.

<sup>4</sup> The octoploid plant described by Darlington (3, p. 216) as *A. volubile latisectum* was actually *A. Wilsoni*.



FIGS. 7-12. Somatic metaphases in Asiatic diploid species of the section *Eu-Aconitum*. Fig. 7, *A. heterophyllum* (dwarf group). Fig. 8, *A. Forrestii*, and Fig. 9, *A. transsectum* (Chinese or paniculate group). *A. transsectum* has the least total chromosome length of any diploid species observed. Figs. 10-12, the three scandent types 29/33, 28/33 and 27/33. The three clones are probably all forms of, or closely related to, *A. Hemslayanum* Pritzl, but are distinct in origin and appearance.  $\times 3,000$ .



FIGS. 13-16. Somatic metaphases in Far Eastern polyploid species of the section *Eu-Aconitum*. Fig. 13, *A. chinense* (tetraploid). Fig. 14, *A. ? palmatum* (Mr. Hay's no. 12, from Nepal);  $2n = 48$  (hexaploid). Fig. 15, 26/30, probably a hybrid derived from *A. palmatum*;  $2n = 45$ . Fig. 16, *A. Wilsoni*;  $2n = 64$  (octoploid). Figs. 13-15,  $\times 3,000$ ; Fig. 16,  $\times 3,200$ . Fig. 16 drawn by C. D. Darlington.

(b) *European Eu-Aconites*.

The following have been examined:

<i>A. paniculatum</i> Lam. (one clone)	$2n = 16$ (see Fig. 13)
<i>A. variegatum</i> L. (three clones)	$2n = 16$ (see Fig. 14)
<i>A. Stoerkianum</i> Rchb. (8 distinct clones, see Appendix 3)	$2n = 24$
<i>A. Napellus</i> L. (13 clones), (see Fig. 15 for a wild plant)	$2n = 32$

In addition the following is of uncertain position :

40 and 44/30, a tetraploid, received from Messrs. T. Smith as '*A. volubile*'; possibly *A. Stoerkianum*. See below.

The classification of the Eu-Aconites has always presented difficulties, owing to variability within the species and plasticity in response to habitat. To this is added a certain amount of confusion due to hybridization.

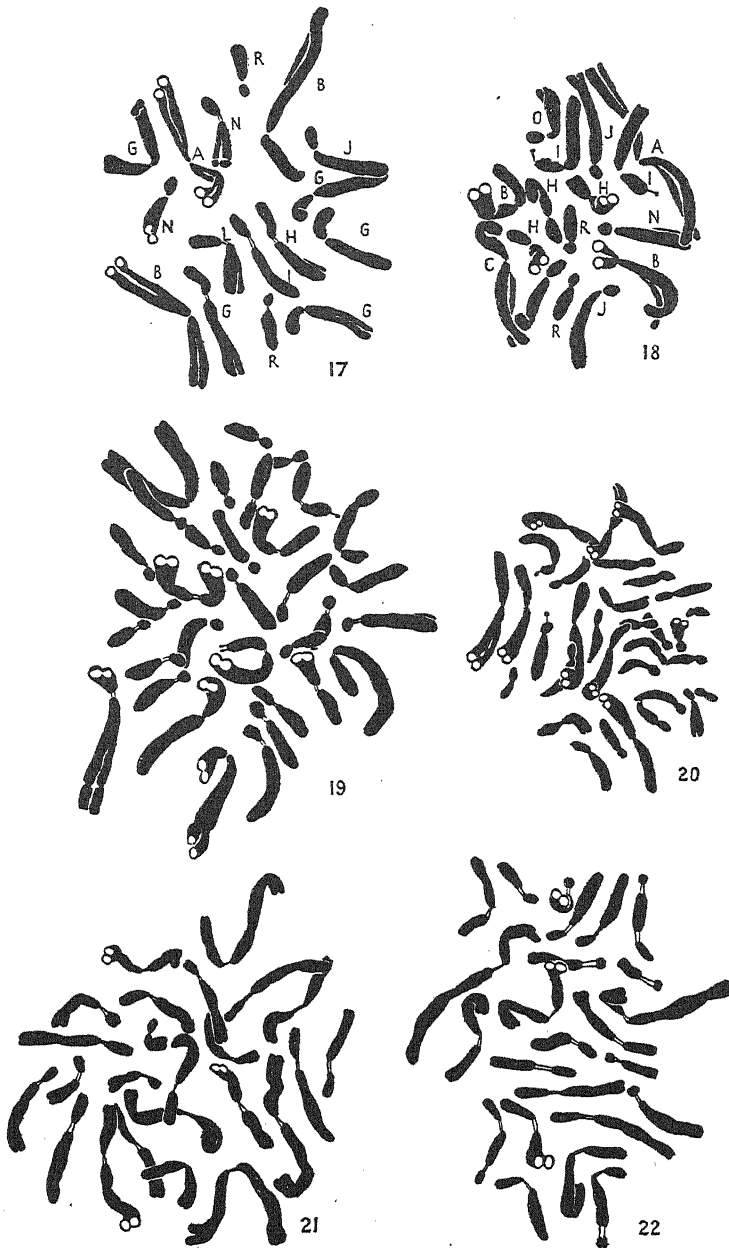
The elder Reichenbach (12) distinguished an inordinately large number of species amongst the European forms. The principal species, however, are four, namely *A. paniculatum* Lam., *A. variegatum* L., *A. Napellus* L., and *A. tauricum* Wulf. The two former belong to Seringe's sub-section Cammarum (DC., Prodrum 1924) and the two latter to the sub-section Napellus. There is further the 'species' *A. Stoerkianum* Rchb., forms of which appear to constitute a large proportion of the summer-flowering Aconites in cultivation.<sup>1</sup> According to the systematists, hybridization takes place between the two sub-sections, and *A. Stoerkianum* is stated to be a hybrid between *A. variegatum*<sup>2</sup> and *A. Napellus*.

It will be seen that the cytological results fully bear out this possibility. The *A. paniculatum* examined was diploid. Three clones of undoubted *A. variegatum* have been shown to be diploid. *A. Napellus* appears to be regularly tetraploid. No less than eight clones, all distinct in origin and appearance, and all placed on systematic grounds in the 'species' *A. Stoerkianum*, proved to be triploid,  $2n = 24$ , as would be expected if *A. variegatum* is diploid and the systematists' view as to the hybrid origin of *A. Stoerkianum* is the true one. The corresponding hybrid between *A. paniculatum* and *A. Napellus* has not been seen by the present authors, but it seems significant that it should have been called by Thomas *A. sterile*, for it should also be triploid.

The situation is complicated by the existence of a tetraploid apparently belonging to the *Stoerkianum* group. This plant, 40 and 44/30, resembles the triploid *Stoerkianum*s in many ways, particularly in the shape of the flower and texture of the leaf. It is, however, extremely tall, over 6 feet even in the dry soil of Merton, where the other clones only grow to about 3 feet; and the individual sprays of flowers are exceptionally long. Nothing can be learnt of its origin except that it was in the herbaceous stock of Veitch's old Coombe Hill nursery, now dismantled. Theoretically it might have come from a cross between an exceptionally tetraploid *A. variegatum* and normal *A. Napellus*; or by the functioning of an unreduced gamete in a cross between normal *variegatum* and *Napellus*;

<sup>1</sup> The aconite, being both decorative and officinal, is evidently a very ancient garden plant, and the actual origin of the various hybrid forms found in different parts of Europe can scarcely be traced.

<sup>2</sup> In the present paper, following Gayer (5), Cammarum is dropped as a specific name, and *A. Cammarum* and *A. rostratum* of various authors are included under *A. variegatum* L.



FIGS. 17-22. Somatic metaphases in European species, all of the section *Eu-Aconitum* except Fig. 20. Fig. 17, *A. paniculatum* (diploid). Fig. 18, *A. variegatum* (diploid). Fig. 19, *A. Napellus* var. *anglicum* (tetraploid). Fig. 20, *A. Anthora* (tetraploid). Figs. 21 and 22, *A. Stoerkianum*; Sparks' Variety and 21/30 respectively (triploid). The chromosomes are smaller in *Anthora* than in the other species examined.  $\times 3,000$ .

Figs. 21 and 22 drawn by C. D. Darlington.

or by abnormal seed setting in a triploid *Stoerkianum*. The question must be left open. It is of course clear that *A. Stoerkianum*, if it can be called a species, is a species of a different nature from *A. Napellus* or *A. variegatum*. It consists of a number of clones, which are probably of independent

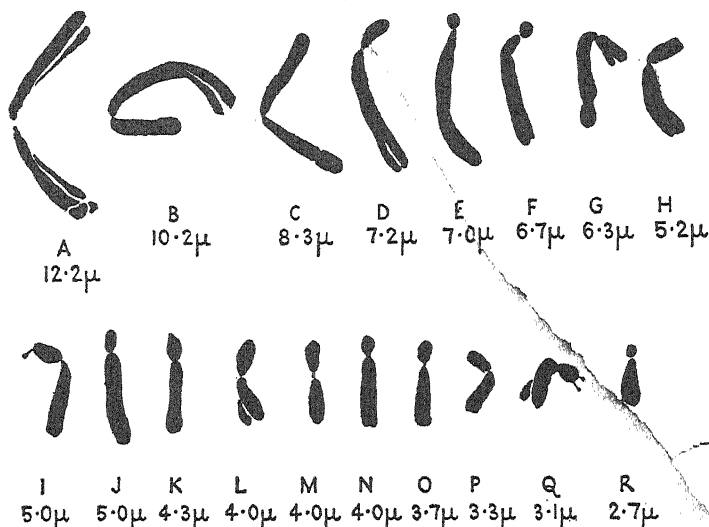


FIG. 23. The chromosome types observed, with the lengths attributed to them.

origin, and some of which, at least, are sterile. These clones have a sporadic distribution; although they may from time to time be discovered in the wild, they are chiefly found, like the *Neo-tulipae* in Europe, in cultivation or as escapes from cultivation.

### *Chromosome Morphology.*

*Aconitum* belongs to the section of Ranunculaceae with large chromosomes. The chromosomes are well differentiated. In the diploids they range in size from  $12\mu$  to a little under  $3\mu$ . In *A. Anthora* and the triploid *Stoerkianum* clone 6/33, three additional small types occur. A few of the chromosomes have median or sub-median attachments, the majority, however, are attached either sub-terminally or about one-third along. The smallest pair in *A. Anthora* are about  $1\mu$  in length, their constriction being sub-median. Two sub-terminal types,  $1.5\mu$  long, are found in 6/33, together with a small terminally constricted chromosome about  $1.1\mu$  long, which has no morphological partner. The chromosomes of *A. Anthora* appear to be all reduced in size.

The attachment constriction, which in most plant species is narrow—often narrower than secondary constrictions—is in most *Aconitum* species



more than one chromatid broad. If this is an artefact it is at least a 'characteristic' one (3).

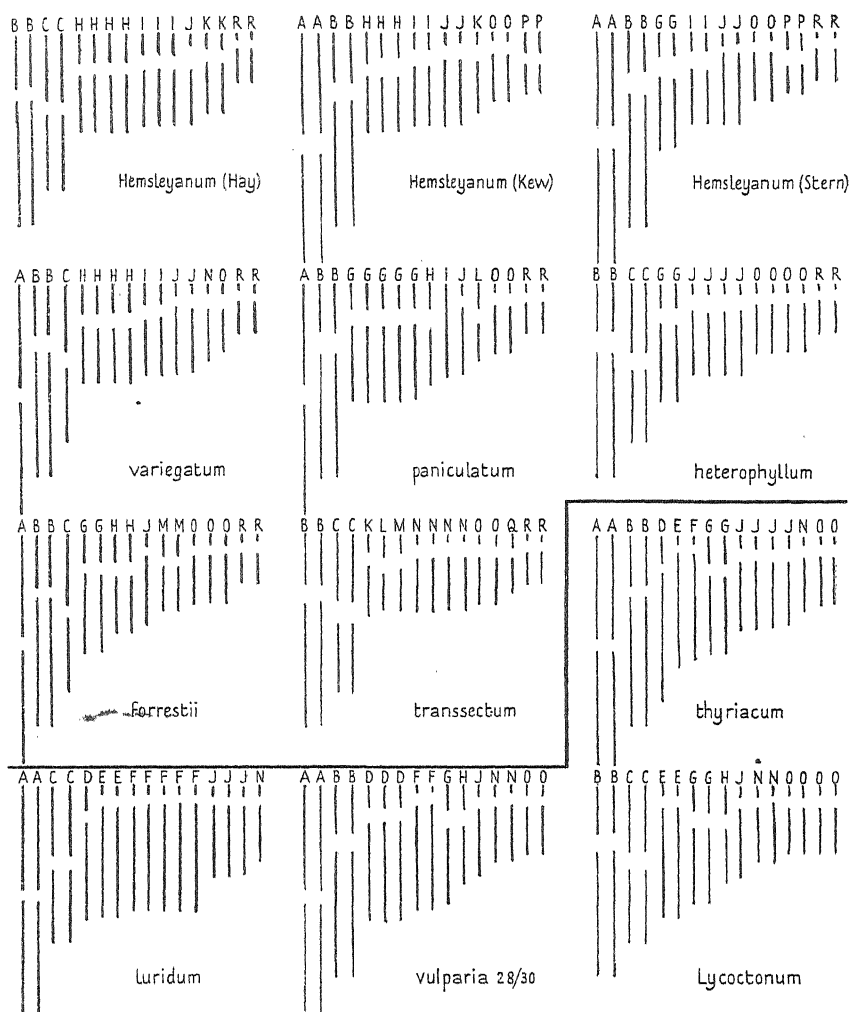


FIG. 24. Complements of 12 forms analysed in accordance with Fig. 23. Section Eu-Aconitum above, Lycopodium below.

Trabants are frequent; as many as four trabanted chromosomes have been seen in one diploid cell. It is quite clear that the nuclei of some clones contain morphologically odd chromosomes, a fact which indicates structural hybridity.

Two diploid species, *A. transsectum* and the *vulparia* clone 21/33, were found to have tetraploid sectors.

An analysis has been made of the chromosome complement of the diploids. The method adopted was to draw all the identifiable types with the camera lucida from individual chromosomes lying flat, and to match these types as nearly as possible from cell to cell. It is not possible to get direct measurements in every case, on account of the chromosomes curling upwards. Only one cell was analysed in each case (the one drawn), and conditions of fixation were not uniform as between the species, so the comparative results here given must be regarded as in the nature of a preliminary survey only.

TABLE I.

*Distribution of Chromosome Types.**Section Lycoctonum.*

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
<i>A. Lycoctonum</i> (20/33) . . .	.	.	2	2	-	2	-	2	1	-	1	-	-	2	4	-	-	-
<i>A. vulparia</i> (28/30) . . .	.	.	2	2	-	3	-	2	1	1	-	1	-	2	2	-	-	-
<i>A. vulparia</i> (39/30) . . .	.	.	2	2	-	-	1	-	2	3	4	-	-	-	1	1	-	-
<i>A. vulparia</i> (21/33) . . .	.	.	1	2	1	2	1	-	1	-	2	2	-	2	-	-	-	2
<i>A. vulparia</i> (17/33) . . .	.	.	1	3	-	4	1	-	2	-	1	2	-	-	2	-	-	-
<i>A. orientale</i> (37/30) . . .	.	.	1	2	1	1	2	-	1	-	5	-	-	1	1	-	-	1
<i>A. luridum</i> (16/33) . . .	.	.	2	-	2	1	2	5	-	-	3	-	-	1	-	-	-	-
<i>A. thyracum</i> (19/33) . . .	.	.	2	2	-	1	1	1	2	-	-	4	-	-	1	2	-	-

*Section Eu-Aconitum.*

<i>A. heterophyllum</i> (24/33) . . .	.	.	-	2	2	-	-	-	2	-	-	4	-	-	-	4	-	2
<i>A. Forrestii</i> (61/30) . . .	.	.	1	2	1	-	-	-	2	2	-	1	-	2	-	3	-	2
<i>A. transectum</i> (64/30) . . .	.	.	-	2	2	-	-	-	-	-	-	1	1	1	2	4	-	1
<i>A. Hemsleyanum</i> Stern (27/33) . . .	.	.	2	2	-	-	-	-	2	-	2	2	-	-	-	2	2	-
<i>A. Hemsleyanum</i> Hay (28/33) . . .	.	.	-	2	2	-	-	-	-	4	3	1	2	-	-	-	-	2
<i>A. Hemsleyanum</i> Kew (29/33) . . .	.	.	2	2	-	-	-	-	1	2	2	1	-	-	2	2	2	-
<i>A. variegatum</i> (22/33) . . .	.	.	1	2	1	-	-	-	4	2	2	-	-	-	1	1	-	2
<i>A. paniculatum</i> (25/30) . . .	.	.	1	2	-	-	-	-	5	1	1	1	-	1	-	2	-	-

The types distinguished, with their calculated lengths, are shown in Fig. 23. The chromosome complements of twelve diploids are shown diagrammatically in Fig. 24 (drawn accurately according to the determined type and calculated length of the type). The complements of sixteen clones are tabulated in Table I. There is considerable variation, but the only difference that can at present be picked out as of diagnostic value is that which marks off the section *Lycoctonum* from the *Eu-Aconites*. It will be seen that in the former group a number of long-medium chromosomes (types D, E, and F) are always present, which are completely lacking in the latter group. The balance is possibly made up on types H and I, though these are not of the same form as D, E, and F. Again the small trabanted chromosomes (type R) are present with great regularity in the *Eu-Aconites*, but almost invariably lacking in the section *Lycoctonum*. The numbers of each type in the two sections (sixteen diploid clones) are

shown in Fig. 25, together with a summary of the same figures grouping the chromosome types (Fig. 26). It will be seen that in spite of the rough

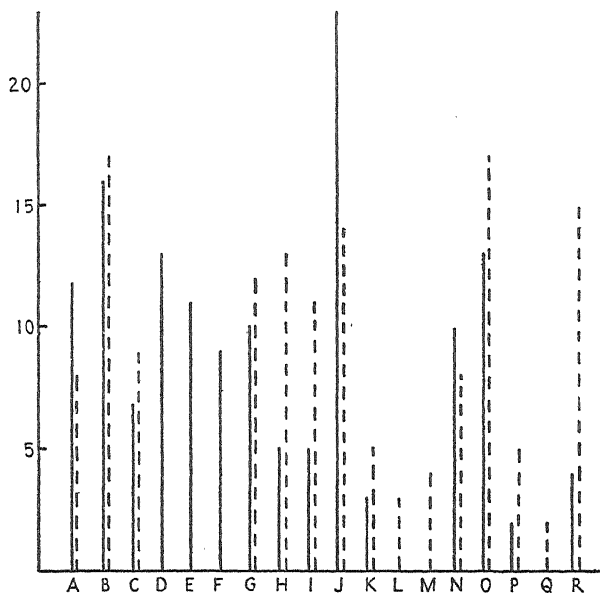


FIG. 25. Sum of the numbers of chromosomes of each type found in 8 members of the section *Lycoctonum* (continuous line) and 8 members of the section *Eu-Aconitum* (broken line).

*Chromosome types.* A, C Long, medium attachment; B long, submedian attachment; D, E, F long-medium, subterminal attachment; G long-medium, attached  $1/3$  along; H, I medium, intermediate attachment; J medium, subterminal attachment; K, L, M, N, O short-medium; P, Q, R short.

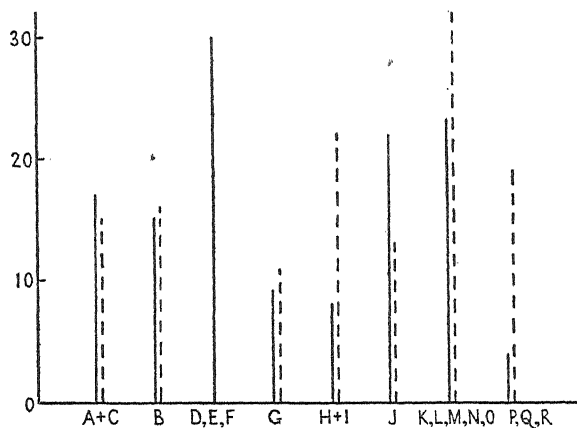


FIG. 26. As in Fig. 25, but with chromosome types grouped.

and ready method of analysis, there is a manifest difference between the sections. This comes out even more clearly in Fig. 27, which shows the total chromosome length. Within the *Eu-Aconites*, so far as can be

discovered at present, the complement appears to give no clue to the systematic position.

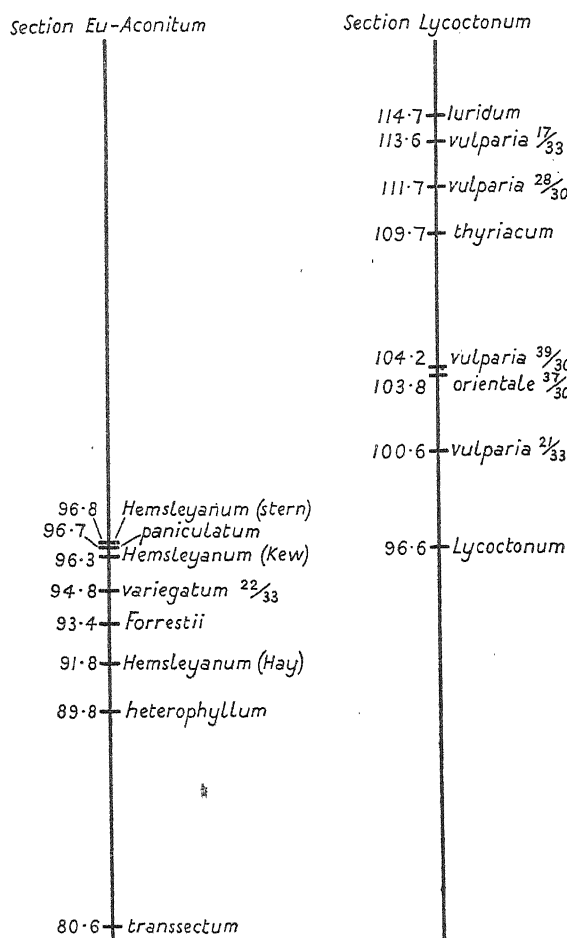


FIG. 27. Total chromosome length as calculated for the complement in 8 forms each of the *Eu-Aconitum* and *Lycoctonum* sections. The mean length is  $92.55 \mu$  in the former and  $106.86 \mu$  in the latter.

Lewitsky (11) has reported on four species of *Aconitum*, including *A. Lycoctonum* and *A. Napellus*. (He had no diploids in the *Eu-Aconitum* section.) He found in his *Lycoctonum* the excess of long chromosomes which we have found to characterize the whole of the *Lycoctonum* section, and remarks on the significant fact that the complement of *A. Lycoctonum* closely resembles that of *Delphinium*.

No attempt has as yet been made to analyse the complement of the polyploid species.

## SUMMARY.

1. Chromosome counts have been made of root-tips in some fifty clones of *Aconitum* from Europe and Asia. There is a single basic number,  $n = 8$ .
2. The section *Lycactonum* appears uniformly diploid.
3. The small section *Anthora* is tetraploid.
4. The section *Eu-Aconitum*, with predominantly blue flowers, contains diploid, triploid, tetraploid, hexaploid, and octoploid forms. The triploids are probably hybrids, as is held by the taxonomists.
5. Variation in chromosome number, as in external morphology, is greatest in the Middle and Far Eastern species.
6. Some account is given of the comparative morphology of the chromosome complements in the diploids. The section *Lycactonum* can be distinguished cytologically from the section *Eu-Aconitum*. Many of the species have complements which cannot be analysed into similar pairs.
7. It appears that polyploidy is the only means by which numerical change has operated in *Aconitum* to give new species.

The authors are greatly indebted to the late Dr. O. Stapf, F.R.S., for guidance in the systematics of the genus. They are also much obliged to A. J. Wilmott, Esq., M.A., for facilities in connexion with the British Museum collections; to their colleague Dr. F. W. Sansome, who handed over the material (under 1930 numbers) originally got together by him for genetical work; and to the following, who have supplied seed or plants: Sir Arthur Hill, K.C.M.G., Sir William Wright Smith, J. W. Besant, Esq., W. Hales, Esq., of the respective Botanic Gardens; E. A. Bowles, Esq., V.M.H., Docent Dr. H. Bruun, Prof. R. Chodat, B. O. Coventry, Esq., Miss A. Eastwood, Prof. M. L. Fernald, T. Hay, Esq., V.M.H., Col. Gavin Jones, Prof. K. Maly, E. M. Marsden Jones, Esq., Prof. B. Nemec.

## APPENDIX I (i).

*Key to the Sections of Genus Aconitum.*

1. Root annual.  
Section *Gymnaconitum* Stapf (*A. gymnandrum*).
2. Root rhizomatous. Flower yellow or purple.  
Section *Lycactonum* DC. (*A. Lycactonum*, *A. vulparia*, *A. orientale*, *A. laeve*, *A. luridum*, &c.).

## 3. Root tuberous.

Sepals persistent after flowering, flowers generally yellow.

Section *Anthora* DC. (*A. Anthora*).

Sepals deciduous, flowers generally blue or purple.

Section *Eu-Aconitum* C. A. Mey. (*A. Napellus*, *A. variegatum*, *A. paniculatum*, *A. Wilsoni*, *A. rotundifolium*, *A. heterophyllum*, *A. Forrestii*, *A. Hemsleyanum*, &c.).

## APPENDIX I (ii).

*Working Classification of the Eu-Aconites.*

(With special reference to the Far East.)

The groups are based on general appearance and must not be taken as strict taxonomic units. They run together, but not so much as to vitiate their usefulness. Pending an adequate monograph, some working classification of the enormous group of Far Eastern species has to be used in handling material.

1. *Dwarf type*. Dwarf mountain forms, rarely above 1 foot. E.g. *A. Hookeri*. (*A. heterophyllum* is rather taller, but belongs here.)
2. *Slender type*. Slender forms with dissected leaves. E.g. *A. delphinifolium*. (Not represented.)
3. *Napellus type*. Forms of medium growth, with dissected leaves and generally narrow inflorescence. E.g. *A. Napellus*, *A. soongaricum*. (Not represented in our Asiatic material.)
4. '*Chinese*' or *paniculate type*. Forms of medium growth, with branched, leafy stems and generally paniculate inflorescence. E.g. *A. chinense*, *A. Forrestii*.
5. *Scrambling type*. Climbing or scandent forms. E.g. *A. Hemsleyanum*, *A. volubile*.

## APPENDIX I (iii).

*Key to the Principal European Eu-Aconites.*

(Omitting the hybrids, lumping all sub-species: largely following Gáyér.)

## 1. Inflorescence lax: seeds ridged on face.

*Sub-section Cammarum.*

Flower-stems glabrous

*A. variegatum.*

Flower stems covered with patent hairs

*A. paniculatum.*

## 2. Inflorescence crowded: seeds smooth on face.

*Sub-section Napellus.*

Flowering stems covered with adpressed hairs

*A. Napellus.*

Flowering stems glabrous

*A. tauricum* (Eastern Europe only).

## APPENDIX I (iv).

*Characters of A. Napellus, A. variegatum, and A. Stoerkianum.*

Both *A. Napellus* and *A. variegatum* are highly variable, but they do not appear to overlap. The following table gives the salient characters of the two species and of *A. Stoerkianum*, the alleged hybrid between them.

<i>A. Napellus.</i>	<i>A. Stoerkianum.</i>	<i>A. variegatum.</i>
Upper part of stems downy	Glabrous or nearly so	Glabrous
Crowded inflorescence, many flowers	Many flowers	Few flowers
Inflorescence leafless, flowers carried well above the leaves	Intermediate	Inflorescence leafy
Often unbranched; if branched, angle of branching narrow	Usually much branched; angle wide	Often unbranched; if branched, angle wide
Short hood and short beak	Hood often tall and strongly beaked	Hood often tall and strongly beaked
Leaves characteristically dissected, large	Leaves very variable	Leaves not greatly divided, rather small.

## APPENDIX II.

*List of Species Examined by Langlet (9, 10).**Diploid*,  $2n = 16$ .*A. excelsum.**A. septentrionale.**A. kamtschaticum* (as '*Aconitum* sp.' in 1927 paper).*A. vulparia.**A. volubile latisectum.**Triploid*,  $2n = 24$ .*A. Napellus.**A. Napellus* Sparks' Variety.*A. variegatum.**Tetraploid*,  $2n = 32$ .*A. californicum.**A. Delavayi.**A. Kuznetsoffii.**A. paniculatum.**A. Anthora.**Octoploid*,  $2n = \text{ca. } 64$ .*A. Wilsonii.*

Langlet reports 'Sparks' Variety' as triploid. He also reports a triploid *variegatum* and a triploid *Napellus*, but from information which he has been kind enough to supply privately, it appears very probable that these plants were garden hybrids of the *Stoerkianum* type so freely represented in our own material. His '*variegatum*', like Sparks' variety, was sterile and set no seed.

Langlet gives no authorities for his names and appears to have taken them from the Botanic Garden labels, so his identifications should not be too much relied upon. His object was a general survey of the Ranunculaceae.

## APPENDIX III.

*List of Plants Examined, with Particulars of Origin.*<sup>1</sup>*Section Lycoctonum* (all diploid,  $2n = 16$ ).*A. vulparia* Rchb. (Lumping the sub-species.)

1. 28/30. From the Chelsea Physic Garden as *Lycoctonum*. Leaves<sup>2</sup> deeply cut, segments broad. Very coarse grower.

<sup>1</sup> Specimens of all the stocks used are being deposited at the Natural History Museum, London. The numbers cited are the record numbers under which the plants have been grown at Merton.

<sup>2</sup> Notes refer to the basal leaves.

2. 39/30. Another clone of the same origin.
3. 34/30. From Kew as *barbatum*.
4. 17/33. From Messrs. Wm. Wells as *Anthora*. Origin unknown, has been in cultivation with them for many years. Leaves variable, hood somewhat triangular.
5. 21/33. From Col. Gavin Jones as *Anthora*. Collected, or raised from seed collected, in the Alps. Leaf segments broad. Short, rather dense raceme.

*A. orientale* Boiss.

1. 37/30. From Messrs. van Tubergen as *orientalis*. Helmet tall and narrow, leaves divided to the base. Dense panicle.
2. 38/30. From Messrs. van Tubergen as *orientalis ochroleucum*. Helmet and leaves similar to 37/30, inflorescence less dense.
3. 48/30. From Kew as *vulparia*. Like 38/30 but leaves cut into very narrow segments.

*A. Lycoctonum* L. (= *A. septentrionale* Koelle). 20/33.

From H. G. Bruun, collected wild in Sweden. Leaves palmately cut, but round in outline; segments very broad. Flowers lilac.

*A. luridum* Hook fil. et Thoms.

16/33. From Kew, where it was raised from seed sent in from Darjeeling. Livid purple flower, tall broad hood. Plant clothed with yellow hairs.

*A. thyracum* Blocki.

19/33. From Glasnevin. Greenish-purple flower, extremely tall narrow hood with a slight crook in it.

*Section Anthora* (tetraploid,  $2n = 32$ ).

*A. Anthora* L.

- 15/33. From Mr. E. A. Bowles, cultivated in his garden.
- 33/33. Bought from Messrs. Thompson and Morgan, as *A. aureum*.

*Section Eu-Aconitum* C. A. Mey.

(a) Asiatic.

i. *Dwarf group* (both diploid,  $2n = 16$ ).

*A. heterophyllum*.

24/33. From Mr. T. Hay, Office of Works, who raised it from seed collected in Nepal. About 18" high, flowers striped blue and white. A common Indian species.

*A. Toppinii* Stapf.

23/33. From Kew. Very dwarf.

ii. *Chinese group* (leafy, paniculate).

Diploid,  $2n = 16$ .

*A. Forrestii* Stapf.

61/30. From Glasnevin.

*A. transsectum* Diels.

64/30. From Edinburgh.

Tetraploid,  $2n = 32$ .

*A. chinense* Sieb.

(1) 22 and 23/30. Messrs. van Tubergen as *Fischeri*. 2-3', autumn-flowering. Leaves shining. Flowers sky blue, on characteristically short branches. Common in the trade as *Fischeri*, a species with which it ought not to be confused.



- (2) 62/30. From Glasnevin as *Fortunei*. Dwarfier than (1), with darker flowers. Identified by Dr. Stapf.

*A. ? spicatum.*

Mr. Hay's no. 414, from Nepal.

Mr. Hay's no. 409.

31/33. From Nepal. Not flowered here.

**Hexaploid,  $2n = 48$  or approximately.**

33/33. Mr. Hay's no. 12, raised from seed collected in Nepal. Possibly *palmatum*.  $2n = 48$ .

26/30. Plants sent from Edinburgh as *palmatum*, and involving that species, but almost certainly hybridized since introduction. Chromosome number was not exactly 48 but varied from 45 to 47.

**Octoploid,  $2n = 64$ .**

*A. Wilsoni* Stapf.

(1) 10 and 16/30. From Edinburgh as *volubile latisectum*.

(2) 17/30. From Kew as *Delavayi*.

(3) 18/30. From the Merton garden.

(4) 27/30. Seedlings, Edinburgh.

The plants vary a little, but their ultimate source is not known and they may all derive from the same introduction.

iii. *Scrambling group.*

**Diploid,  $2n = 16$ .**

*A. ? Hemsleyanum* Pritzel.

(1) 27/33. As *volubile* from Major Stern at Goring by Sea, who had it from a Forrest expedition. (Major Stern in 1932 supplied herbarium material and later tubers. The tubers gave  $2n = 16$ . In 1933 on being asked for more material he sent another and distinct clone. Presumably they are both from the same collecting.)

(2) 28/33. As *volubile* from Mr. Hay, who raised it from seed collected near Gantock in Sikkim. A very strong and freely branched climber. Pale blue flowers.

(3) 29/33. As *volubile* from Kew, who have had it in cultivation for many years but do not know its origin. A slender climber. Earlier in flower than the two preceding.

The above three forms, 27, 28, and 29/33, though distinct in appearance and origin bear a general resemblance to one another. They may tentatively be placed under:

*A. Hemsleyanum*.

**Tetraploid,  $2n = 24$ .**

*A. ? volubile* Pall.

8/33. Received from Glasnevin as *volubile*. Very distinct from the three clones above. Flowers similar to those of *A. chinense*. This is probably the Siberian plant originally described as *volubile* by Pallas. Glasnevin could not give the origin of their stock.

iv. *Presumed hybrid.*

**Triploid,  $2n = 24$ .**

56/30. From Kew as *uncinatum*, and probably a hybrid involving that species.

## (b) European.

i. *Sub-Section Cammarum*.Diploid,  $2n = 16$ .*A. paniculatum* L.25/30. From Kew as *cernuum* (a sub-species of *paniculatum*).*A. variegatum* L.

22/33. Plant about 2 feet high, few-flowered, leaves small and dry looking (not juicy). Cultivated on the rock-garden at Kew and identified by Dr. Stapf. Flowers blue and white.

7/33. Collected by C. D. Darlington near the top of the Halltal in Austria (Salzkammergut). Flowers blue.

9/33. From Professor Nemec at Prague as *rostratum*. Collected from the wild. Flowers blue. Rather more robust than the two preceding clones.

*A. variegatum* is not easy to obtain, except by collecting in the wild; the plants offered by nurseries are almost always triploids, i.e. *A. Stoerkianum*.

ii. *Sub-section Napellus*.Tetraploid,  $2n = 32$ .*A. Napellus*.(1) 59/30. From Kew as *Forrestii*. A central type.(2) 2/30. From Messrs. T. Smith. Determined by Dr. Stapf as var. *neomontanum*.(3) 63/30. From Edinburgh as *paniculatum*. Similar to (2).(4) 25/33. From Professor Chodat at Geneva, as *Napellus*.(5) 26/33. From Professor Chodat, as *variegatum*.(6) 2/33. From Professor Nemec at Prague, as *Napellus*.(7) 3/33. From Professor Nemec, as *rostratum*. Weak stem, evident tendency to scramble.(8) 1/33. Var. *anglicum*. From Mr. E. M. Marsden-Jones. Wild at Edington, Wilts., on the site of an old monastery.(9) 31/30. Var. *anglicum*. From the Cambridge Botanic Garden, as *anglicum*. Agrees closely with 1/33. These two clones are in flower nearly a month before the rest.(10) 43/30. From Kew as *volubile*.(11) 11 and 12/30. From Messrs. T. Smith as *pyramidale*.(12) 13/30. From Messrs. van Tubergen as *Napellus* var. *carneum*.(13) 50 and 54/30. From Messrs. T. Smith as *giganteum album*. White flowers.iii. *Presumed hybrids* (= *A. Stoerkianum* Rchb.).Triploid,  $2n = 24$ .(1) 3 and 4/30. From Messrs. van Tubergen as *bicolor*. Flowers blue and white.(2) 5 and 6/30. From Messrs. van Tubergen as *Napellus*, Sparks' variety. Dark blue. (Was introduced by Messrs. Maurice Pritchard circa 1898, being obtained from a cottage garden in Hampshire and named after a gardener called Sparks in Messrs. Pritchard's employment.)

(3) 20/30. From Kew. Flowers blue.

(4) 21/30. From Kew as *Carmichaelii*. Finely cut leaf, stems almost unbranched.

- (5) 41/30. From Kew as *rostratum judenbergense*. Clear deep blue, inflorescence lax; lateral branches long.
- (6) 42/30. From Kew as *rostratum coeruleum*.
- (7) 5/33. Received from Glasnevin as *rostratum*.
- (8) 6/33. Received from Col. Gavin Jones as *paniculatum*. Similar to (2).

Tetraploid,  $2n = 32$ .

40 and 44/30. As *volubile* from Messrs. T. Smith, of Newry, who had it from Veitch's at Coombe Hill. Mr. G. Harrow, a former manager of this nursery, who has kindly looked through the records of Wilson's introductions, can find no mention of this plant. It may be Asiatic but is more probably European in origin.

All the *Stoerkianum* clones were glabrous or nearly so, like *variegatum* and unlike *Napellus*.

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# A Phytogeographical Problem: Fossil Plants from the Kerguelen Archipelago.

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With Plates XIV and XV, three Maps, and four Figures in the Text.

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## INTRODUCTION.

THE plants described in this paper were discovered by Dr. Aubert de la Rüe in March 1931 on his second visit to the Kerguelen Archipelago. In the course of an earlier geological exploration (1928-9) he found beds of lignite and carbonized wood but neither petrifications nor impressions. The specimens were submitted to Professor Carpentier and Professor Depape, and at the suggestion of the former they were then sent to Cambridge. I was asked in the first instance to furnish brief notes, and later to examine the material in more detail: the notes, together with information supplied by my French colleagues, were utilized by Dr. de la Rüe in a short palaeobotanical summary included in his recent book, 'Étude géologique et géographique de l'Archipel de Kerguelen' (53, p. 145). Most of the better specimens were photographically illustrated but without descriptions of the actual fossils.

I am greatly indebted to Dr. de la Rüe for the opportunity of examining his collection and for his generosity in placing the material at my disposal. I am further indebted to him for herbarium sheets of recent plants, and photographs illustrating geological features. The figured specimens, with the approval of their discoverer, have been given to the British Museum.

Though much of the material is fragmentary and inadequate for precise identification, it is important as providing more convincing evidence than has hitherto been available of the former existence of trees on a land that is now treeless. More than ninety years ago Sir Joseph Hooker (33 ; see also 18) recorded the occurrence of prostrate trunks of fossil trees of no mean girth, one of them over 7 ft. in length, in many of the basaltic sheets which are the dominant feature of the Archipelago ; he referred also to incinerated remains of stems that had been swallowed up by the lava flows. The petrified and carbonized wood has generally been regarded as proof of the former presence of mixed coniferous forests on the island : now for the first time Araucarian foliage-shoots, seminiferous cone-scales, a few leaves of flowering plants, fragments of fern fronds, and other remains fully confirm the former existence of trees and species of smaller plants which are no longer represented in the flora. Petrified wood alone is often untrustworthy as evidence of the growth of trees *in situ* : the more recent discovery shows that the fossils are relics of an autochthonous vegetation. The old problem of the origin of the flora of this remote Archipelago is raised afresh by the material now available.

#### THE GEOLOGICAL AND PHYSICAL FEATURES OF THE KERGUELEN ARCHIPELAGO.

The Kerguelen Archipelago (for a general account see 6, 34, 44, 53, 61, 73, 82), a French possession, in the centre of the Indian Ocean, lies between 48°, 27' and 50° S. lat. ; 68°, 27' and 70°, 35' E. long. ; it is approximately 80 miles long from north to south and slightly broader. It is rather more than 1,000 miles from Enderby Land and Kemp Land on the edge of Antarctica : Heard Island, its basaltic cliffs 6,000 ft. above sea-level, is 300 miles to the south. Sir Douglas Mawson (30) confirmed the occurrence of the 'Gaussberg Rise', which stretches towards the Antarctic continent as a narrow submarine platform (350 fathoms) and is separated from the mainland by water 3,000 metres in depth. The islands of St. Paul and Amsterdam (see Map II, p. 728) are 800 miles to the north-east of Kerguelen. South Africa is 2,500 miles distant. At intervals in the zone of westerly winds stretching 3,120 miles to Cape Horn there are groups of lava-built islands affording evidence of former volcanic activity ; first the Crozets, their precipitous cliffs towering 5,000 ft. above sea-level, 1,200 miles

away, followed by Marion and Prince Edward Islands based on a submarine platform. Farther west is South Georgia, one of a series of islands forming a looped festoon curving eastward from the Falkland Islands and bending back through the south Sandwich group, the south Orkneys to



MAP I.—Sketch-map of the Kerguelen Archipelago: I, II, localities where fossil wood was found by earlier explorers. R. Mt. Ross. J. Peninsule Joffre. III. Port Jeanne d'Arc, where the recent collections were made.

the south Shetlands on the flanks of Graham Land. It has often been suggested that this looped festoon marks the position of a former bridge joining South America and Antarctica: if it does, the lost land lies in places at least 3,000 metres below sea-level. Similarly, if the Crozets are relics of a land-bridge the foundered link between them and Kerguelen lies 4,000 metres deep; and over the ridge between the Crozets and Marion Island soundings reveal a depth of more than 2,000 metres.

Macquarie Island, 600 miles south of New Zealand, from which it is separated by ocean depths of 3,000 fathoms, is linked floristically with the sub-antarctic islands to the west of Kerguelen.

Subsequent to its discovery in 1772 by the Breton Navigator, Yves de Kerguelen, who was followed in 1776 by Captain Cook, the Archipelago has been visited on several occasions by expeditions from different countries. Since Hooker and other members of the Ross Expedition

explored parts of the Archipelago, various additions have been made to our knowledge of the natural history and geology: the recent work of Dr. de la Rüe is an especially notable contribution to a better understanding of the structural features. The terraced hills of basalt (Pl. XIV, Fig. 1) bear a striking resemblance to those of Iceland, the Faroe Islands, and the Inner Hebrides: the Archipelago is a volcanic plateau sculptured by intensive erosion into gloomy hills, culminating in Mt. Ross, over 6,000 ft. above sea-level, escarpments with horizontal dip-slopes, and steep-sided valleys. Though corresponding in latitude with the southern part of England, Kerguelen Land is comparable in barrenness and in its partial covering of ice with Greenland and Spitsbergen: one-sixth of the surface is hidden by ice-fields and glaciers. Widespread mountains and erratics show that formerly almost the whole was glaciated. Dr. de la Rüe, confirming and extending discoveries by other geologists, describes many igneous rocks other than basalts; he speaks of trachytes and andesites as widely spread, of syenites, diorites, and gabbros as playing an important role: granite and other acidic rocks are of rarer occurrence. Mt. Ross is mainly composed of andesitic lava. He recognizes four series of lavas and other igneous rocks: the oldest rocks are believed to be Mesozoic or even earlier; the second series is represented by the more acidic rocks—trachytes and phonolites—probably Eocene in age; the main mass of basaltic sheets, reaching 1,000 metres in thickness, he attributes to Tertiary fissure-eruptions. The last volcanic phase, at the end of the Pliocene stage and continuing into the earlier part of the Quaternary period, is represented by the andesites of Mt. Ross (Map I, R). There are now only a few fumaroles and warm springs. Dr. de la Rüe found beds of volcanic ash resorted by torrential streams and comparatively thick deposits of river-borne sand and conglomerate: it was in these beds of ash and other sediments interstratified with the third (Tertiary) series of lava-flows that the fossil plants were discovered near Port Jeanne d'Arc (Map I, J. III). He found no sedimentary continental rocks.

In a recent lecture before the Geological Society of London Sir Douglas Mawson (42) referred to the occurrence of fragments of a *Globigerina* ooze in beds of tuff on Kerguelen and other islands, indicating that the igneous outbursts had broken through pelagic sediments: he also spoke of the occurrence of plutonic rocks in the south-western part of Kerguelen. He was inclined to assign the lignites incorporated in the fluvial conglomerates resting on the lavas to a late Oligocene age.

It is clear that the Kerguelen rocks are by no means exclusively of volcanic origin; some are coarser-grained, deeper-seated products of crystallization. De la Rüe speaks of the more acidic rocks as part of the Sial, that is the lighter layer, including the continental blocks, which rests on the heavier Sima: he envisages the Archipelago as a platform con-



sisting of a sheet of Sial floating on the Sima and penetrated by fissures through which portions of the substratum welled up as basaltic sheets. The Sial sheet, he thinks, formed part of the Antarctic continent, which in the early Tertiary period enjoyed a temperate climate and was attached to South America and Australia, from which it separated at the beginning of the Eocene period.

On this wind-swept land, where it has been said there is 'the greatest persistence of heavy winds (from the west) of which we have any knowledge' (76), there is very little difference between summer and winter temperatures—not more than 6°: the mean annual temperature is 3° C., approximately that of Iceland. Fogs are almost constant, and the number of sunny days may be only three or four in a month. The low summer temperature and the small amount of concentrated sunshine are the main factors responsible for giving to these mist-shrouded islands a climate much less favourable than the geographical position would lead one to expect.

#### THE PRESENT FLORA OF THE ARCHIPELAGO.

Botanically, Kerguelen Land (for list of plants see 30, 33, 34, 37, 47, 61) is part of a circumpolar province including the sub-antarctic islands to the west and Macquarie Island far to the east, New Zealand with Auckland and Campbell Islands, Fuegia, and the Falkland Islands. A few of the plants are almost cosmopolitan, e.g. *Cystopteris fragilis*, *Hymenophyllum peltatum*, *Polypodium vulgare* (represented by a variety), *Lycopodium Selago*, *Callitriche antarctica*, *Limosella aquatica*, and *Montia rivularis*: these are almost certainly immigrants from the northern hemisphere. One of the most abundant plants is a species of the southern hemisphere Umbelliferous genus *Azorella*, *A. Selago*, which forms enormous cushions: this species has its counterpart in *A. caespitosa* in Fuegia. There are five grasses, including *Poa Cookii*, confined to Kerguelen, the Crozets, and Marion Island, and *Aira antarctica* recorded also from the Crozets, South Georgia, the Falkland Islands, and as far south as Graham Land. *Colobanthus kerguelensis* and *Lyallia kerguelensis* are endemic species, the latter the sole representative of a genus allied to *Pycnophyllum* of the Andes; *Ranunculus Moseleyi*, one of four species of the genus, is another endemic. The Kerguelen cabbage (*Pringlea antiscorbutica*), a favourite resort of lazily crawling wingless flies, occurs also in the Crozets and Marion Island. As Hooker pointed out, the flora is most closely allied to that of Fuegia, though there are twelve species common to Kerguelen and the New Zealand region. The two species, *Uncinia compacta* and *Cotula plumosa*—the only bright colour in a sombre company—occur in New Zealand but not in Fuegia. *Uncinia* is a southern hemisphere genus and *Cotula* is mostly southern. It is noteworthy that with the exception of

*Polypodium vulgare* there is practically no link between the vascular plants of Kerguelen and South Africa.

Half of the Lichens and 30 per cent. of the Mosses are said to be Arctic species ; most of the sixty species of Mosses are believed to be endemic. The Characeae are represented by *Nitella antarctica*.

#### DESCRIPTION OF THE FOSSIL PLANTS.

##### *Bacillariaceae* (Diatoms).

An investigation by Miss Conway of crushed pieces of rock treated with acid and caustic potash revealed the presence of several Diatoms. Professor Fritsch, to whom the material was sent, tells me that after a preliminary examination he found *Fragilaria bicapitata* A. Meyer, a species recorded from sub-alpine habitats, other species of the genus, also examples of *Cymbella*, *Diatoma*, *Gomphonema*, *Achnanthes*, and *Navicula*. Professor Fritsch very kindly adds that he will examine the slides in more detail and, if specific identification is possible, embody the results in a separate paper.

##### *Muscineae*.

*Dicranites australis* Dix., Pl. XV, Fig. 28. Dr. H. N. Dixon (14) has published an illustrated description of two Mosses collected by Dr. de la Rüe, *Dicranites australis* and *Muscites thuidioides*. The material is admittedly too incomplete to be assigned to an 'exact generic position': external characters of sterile fragments are alone available. The type-specimen of the *Dicranites*, reproduced approximately twice natural size in Pl. XV, Fig. 28, shows the second tips of branches covered with falcate or sub-circinate leaves. Though not necessarily most closely allied to the genus *Dicranum*, it is undoubtedly a Moss of the Dicranoid type, a type still represented in the Archipelago.

There are many fragmentary remains scattered over the shale, such, for example, as that shown in Pl. XV, Fig. 23, which are probably Mosses, but cannot be determined: it is clear that the conditions were favourable, as they are now, for the growth of representatives of this group.

##### *Filicineae*.

The collection includes fragments of more than one type of Fern, all of which are sterile and inadequate as guides to affinity.

*Filicites*, sp. A, Pl. XIV, Fig. 6 ; Pl. XV, Figs. 29, 30.

'Fern'. De la Rüe (53), Pl. XXV, Fig. 11.

The largest specimen is reproduced as Pl. XIV, Fig. 6 ( $\times 2\frac{1}{4}$ ): it appears to be a piece of a bipinnate frond ; some of the smaller pinnules, all

of which are very imperfectly preserved, are more or less deltoid, and basally contracted, while others are slightly lobed and attached by a broader base. It does not agree closely with any of the few Ferns in the present flora, but may be compared with *Aspidium mohrioides* Bory (33, Pl. 149), a species which occurs in the Falkland Islands, Tierra del Fuego, S. Georgia, the Auckland Islands, and elsewhere. It is, however, impossible to attempt a reference even to a generic position. The pieces of pinnae shown in Pl. XV, Figs. 29, 30, may be specifically identical with the larger specimen: traces of venation are visible in some of the pinnules.

*Filicites* sp. B. Pl. XV, Figs. 31, 32. The relatively broader and more rounded pinnules shown in Pl. XV, Fig. 32, and the single pinnule (Pl. XV, Fig. 31) remind one of some species of *Gleichenia*, a genus not now represented in the Kerguelen flora.

Pl. XV, Fig. 33 (de la Rüe (53), Pl. XXV, Fig. 9). This shrivelled specimen resembles that represented in Pl. XV, Fig. 32, in the more open arrangement of the pinnules; it is of no value as a record.

*Filicites* sp. Pl. XV, Fig. 27. This little specimen ( $\times 3\frac{1}{2}$ ) is probably part of a very young plant; imperfect pinnae converge to a disk-like carbonized piece of rhizome.

While some of the fern fragments may be compared with the widely distributed sub-antarctic species *Lomaria alpina*, scraps such as those shown in Pl. XIV, Fig. 6, and Pl. XV, Fig. 32, afford evidence of the former existence of ferns of a type no longer represented in the Kerguelen Archipelago.

### *Coniferales.*

*Araucarites Ruei* sp. nov. Pl. XIV, Figs. 2-4, 8, 9, 13, 14; Pl. XV, Figs. 21, 25; Text-fig. 2.

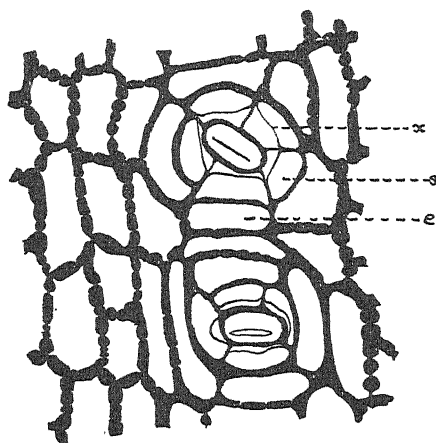
*Araucarites* de La Rüe (53), Pl. XXIII; Pl. XXIV, Figs. 1-10, 13.

After some hesitation, foliage-shoots bearing leaves of two sizes are referred to a single species in which are included detached seed-bearing scales. Unfortunately it has not been possible to make preparations of the cuticles of the smaller leaves, but only of the larger. The close agreement in form of all the leaves, and the occurrence of specimens intermediate in size between such specimens as those shown in Pl. XIV, Figs. 4, 8, and 20, are points in favour of specific identity. It is, however, by no means impossible that, as others (53) have suggested, more than one Araucarian conifer flourished in the Archipelago.

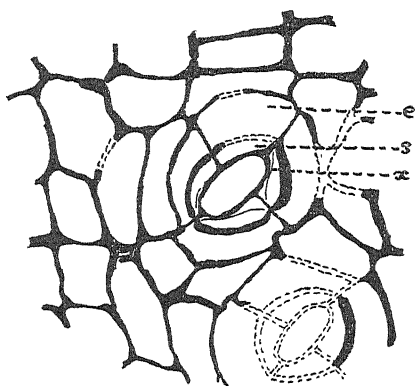
*Foliage-shoots.* Examples of the larger form are shown in Pl. XIV, Figs. 2-4: the leaves have a maximum breadth of 7 mm. and are 1.2 cm. long; they are broadly triangular and falcate; the apex is more obtuse than acute. In some examples there is a fairly well-marked median keel.

Preparations of carbonized films made and described by Miss Conway

reveal characters agreeing with descriptions by Dr. Florin (22) of the cuticles of recent species included in the section Eutacta, particularly *Araucaria excelsa*. In the cuticle of *A. excelsa* represented in Text-fig. 1, subsidiary cells (*Nebenzellen* of Florin) are seen at *s*, and an encircling cell



TEXT-FIG. 1.



TEXT-FIG. 2.

TEXT-FIGS. 1 and 2. Fig. 1. Cuticle of a leaf of *Araucaria excelsa*; *e*, encircling cells; *s*, subsidiary cells; *x*, margin of cutin.  $\times 300$ . Fig. 2. Cuticle of *Araucarites Ruei*.  $\times 300$ . (Prepared from a specimen like that in Pl. XIV, Fig. 4.)

(*Kranzzell* of Florin) at *e*; the single line *x* is interpreted as the free inner margin of the cutin which extends deeply into the wall separating the subsidiary and encircling cells. In the imperfectly preserved cuticle from one of the large-leaved fossils shown in Text-fig. 2, the dotted lines mark the inferred position of portions of walls which have been partially obliterated. Comparing the two figures, one notices that the only real difference appears to be the absence of pitting in the fossil walls, a contrast possibly depending upon the state of preservation.

In the smaller form of shoot (Pl. XIV, Fig. 9, Pl. XV, Figs. 20, 25) the leaves, as seen both in profile and in surface-view, differ only in size from those of the larger specimens.

*Cone-scales.* The scales reproduced in Pl. XIV, Figs. 13 and 14, are preserved in a fine-grained ash; they are 1.5 cm. broad at the distal end and taper to a narrow base. The broadly rounded outer edge of the flattened margin on the scale at the lower right-hand corner of Pl. XIV, Fig. 14, is flanked by two lobes which form part of the thin lateral wing. A median longitudinally ridged region marks the position of the seed: in some specimens the seeds are preserved as carbonized detachable bodies. In the two examples reproduced on a larger scale in Pl. XIV, Fig. 13, the relatively long upturned spinous process is clearly shown.

Though a superficial comparison of the larger foliage-shoots with recent species might dispose one to select *Araucaria Rulci* as the nearest type, Miss Conway's examination of the cuticle indicates a closer resemblance to the Norfolk Island *A. excelsa*. The cone-scales agree generally with those of *A. excelsa*, *A. Cookii*, and allied species. Apart from the question of deciding which living *Araucaria* is the nearest relative of the Kerguelen conifer, the connexion is with species in the Australian-New Caledonian region and not with those of South America.

*Wood of conifers.* Dr. de la Rüe obtained many pieces of wood preserved as lignite: one of the larger examples is illustrated in his book (53), Pl. XXIII, Fig. 1). Such of the material as was examined microscopically in this laboratory did not furnish any new facts. We already know of the occurrence of wood with the *Araucarian* and the *Cupressinoxylon* types of structure (18, 67).

#### *Conifer Shoots of Uncertain Affinity.*

*Elatocladus kerguelensis* sp. nov. Pl. XIV, Fig. 5, Pl. XV, Figs. 18, 18A.

*Elatocladus.* De la Rüe (53), Pl. XXIV, Fig. 11.

In the note on the Kerguelen fossils in Dr. de la Rüe's book, some scraps of foliage-shoots are figured as examples of *Elatocladus*, the generic name usually employed for certain forms which in the absence of reproductive organs and through lack of cuticles cannot be assigned to a precise position.

The impressions shown in Pl. XIV, Fig. 5 and Pl. XV, Fig. 18 ( $\times 2$ ), differ in the straighter and relatively narrow leaves with decurrent bases (Pl. XV, Fig. 18A) from the *Araucarites*. Some of the leaves show signs of a mid-rib. Comparison may be made with *Sequoia sempervirens*: one can only suggest the possibility of a connexion of such shoots as these with the stem previously assigned on anatomical evidence to the comprehensive genus *Cupressinoxylon* (18).

*Elatocladus* sp. Pl. XIV, Fig. 11. This fragment, distinguished by the very narrow and relatively long leaves, may be a young shoot of an *Araucaria*. It closely resembles the juvenile form of some recent *Araucarian* species, though one cannot be sure that it is a conifer.

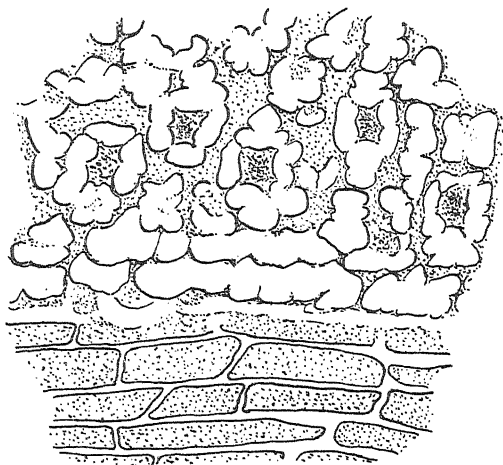
#### *Plantae incertae sedis.*

*Desmiophyllum* sp. a. Pl. XV, Fig. 26.

'*Monocotyledon*'. De la Rüe (53), Pl. XXV, Fig. 5.

The generic name *Desmiophyllum*, established by Lesquereux and subsequently revived as a convenient designation for linear leaves of uncertain position, is adopted in preference to *Monocotylophyllum* for the specimen

reproduced in Pl. XV, Fig. 26, because of the characters of the cuticle (cf. Text-fig. 3). Superficially the two carbonized strips resemble broadly linear leaves of some Monocotyledon, which one would expect to be connected with a common rhizome. A more thorough examination showed



TEXT-FIG. 3. Cuticle of *Desmiophyllum* sp. a.  $\times 300$ .

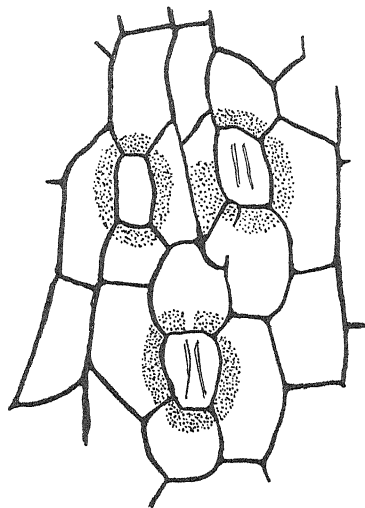
that the numerous longitudinal veins are not strictly parallel as they would be in a single leaf, and preparations of the thin carbonized film covering the impression revealed the presence of more than two layers of cuticle. The apparently broad leaves appear to be made up in part at least of matted masses of narrow leaves.

The cuticle represented in Text-fig. 3 was obtained by Miss Conway from an imperfectly preserved linear leaf 4 cm. long and 0.4 cm. broad, with a mid-rib and about four parallel and more slender veins on each side. On one surface of the cuticle no stomata were found; on the other they occur in longitudinal rows, and are surrounded by subsidiary cells which, with the adjacent epidermal cells, are strongly papillose.

Stomatal bands of similar structure occur on the cuticles of the specimen shown in Pl. XV, Fig. 26. The bands are usually about fifteen cells in width and separated by the same number of epidermal cells. In general appearance the cuticle (Text-fig. 3) recalls that of *Taxus baccata*, but the venation and leaf-form are of a very different type. It has not been possible to match the form and structure of the fossils with any recent Angiosperm, nor with leaves of the extinct genus *Podozamites*. The character of the cuticle by no means excludes a Gymnosperm relationship. Despite the fact that no satisfactory comparisons with recent plants can be made, the structure is worth recording if only as an illustration of the danger of being misled by external form.

The three following specimens are simply designated *Desmiophyllum* sp.; proof of identity with that already described is lacking.

Pl. XIV, Fig. 7. 'Monocotyledon'. De la Rüe (53), Pl. XXV, Fig. 7. These linear leaves with five or six parallel veins are preserved as dark



TEXT-FIG. 4. Cuticle of *Phyllites kerguelensis*.  $\times 300$ .

brown stains on a lighter ground: they may belong to some Monocotyledon or they may be leaves similar in cuticle-structure to those already described.

*Desmiophyllum* sp. Pl. XV, Figs. 16, 16A. Fragments of cuticle obtained from this tapered linear leaf were too incomplete to furnish any clear indication of affinity.

Pl. XV, Fig. 17. This is part of a leaf 3.5 mm. broad: on each side of a rather broader vein there are four more slender veins; it is indeterminate.

*Phyllites kerguelensis* sp. nov. Pl. XIV, Fig. 10.

'Monocotyledon'. De la Rüe (53), Pl. XXV, Fig. 6.

This incomplete piece of leaf shows slightly more than half the original breadth of the lamina; there is a fairly prominent mid-rib near the left-hand edge and numerous parallel veins are faintly seen on the carbonized surface. Miss Conway has supplied the following description of the cuticle (Text-fig. 4). The leaf is amphistomatic; stomata occur in bands parallel to the mid-rib; each band is four to eight cells in breadth and separated from adjacent bands by seven to ten rows of epidermal cells. The stomatal structure, while resembling that found in many Monocotyledons, does not agree with that of the Gramineae and Cyperaceae as described by Molisch

(43), nor with the stomatal characters of the Commelinaceae. There is also a similarity to the structure of some Gymnosperms. It would seem from the classification given by Solereder (72) that there is no very definite stomatal distinction between Gymnosperms and Monocotyledons. The form of the leaf and the venation are unusual among Gymnosperms; the leaves of a few species of *Podocarpus* of the section *Eupodocarpus* are comparable, though the fossil leaf is slightly broader than those of the living plant.

*Leaves of Dicotyledons.*

*Dicotylophyllum Edwardsi* sp. nov. Pl. XIV, Fig. 12; Pl. XV, Fig. 22.  
'Dicotyledon.' De la Rüe (53), Pl. XXV, Fig. 1.

The best specimen of dicotyledonous leaf, named after Mr. Edwards of the British Museum, is that shown in Pl. XIV, Fig. 12; it is an impression of an obcuneate leaf, 2.5 cm. long, with a maximum breadth of 1.5 cm. There are a few shallow indentations on the imperfectly preserved margin of the broadly rounded apical part, but the lamina is mainly entire. From a relatively prominent mid-rib a few secondary veins are given off, the lowest of which follows a marginal course. The lamina tapers gradually to the base. None of the existing Kerguelen plants have this type of leaf: in shape and in venation it is comparable with some species of the Rubiaceous genus *Coprosma*, a genus which has its chief centre in New Zealand and the islands to the south. It is, however, hardly possible to assign it to a generic or even to a family position.

Pl. XV, Fig. 21. ('Dicotyledon'. De la Rüe (53), Pl. XXV, Fig. 2.) This smaller leaf may be specifically identical with the larger, though the impression suggests a thicker and more leathery type.

*Dicotylophyllum* sp. α. Pl. XV, Fig. 24. This small and imperfectly preserved obcuneate leaf with a strongly dentate upper margin reminds one of the lobed leaves of two of the existing Kerguelen plants, *Ranunculus trullifolius* and *Azorella Selago*; the venation is very incompletely shown.

*Dicotylophyllum* sp. β. Pl. XIV, Fig. 15; Pl. XV, Fig. 19. This is a piece of a larger leaf in which the venation is of a different pattern. Only the stronger veins are seen as grooves dividing the lamina into irregular and slightly raised areas.

Pl. XIV, Fig. 15 (×4) represents one of several torn fragments preserved on a different kind of rock which are interpreted as the remains of still larger leaves exhibiting a reticulation formed by the veins as in Pl. XV, Fig. 19. The clean-cut semicircular area cut out of the piece in the middle of Pl. XIV, Fig. 15 reminds one of the work of a leaf-cutting bee.

Fragmentary as are most of the fossils they are worth recording as evidence of the former existence on the Archipelago of plants other than



those which compose the present flora. By far the most interesting specimens are those of *Araucarites*. The fossil plants do not of themselves enable us to assign the sedimentary beds to a definite geological position. The occurrence of a few types which are in all probability extinct is a point in favour of a Tertiary rather than a Quaternary age, a conclusion consistent with the indecisive geological evidence.

#### THE GEOGRAPHICAL DISTRIBUTION OF THE GENUS *ARAUCARIA*.

##### (i) *Present*; (ii) *Past*.

##### (i) *The Present Distribution*.

The genus *Araucaria* (24, 49) is now represented by about fifteen species, six of which are assigned to the section *Colymbea* and nine to the section *Eutacta*: in the typical *Colymbea* type the absence of wings on the seed-scales is one of the sectional characters, but in three species endemic to New Guinea the flat and comparatively long leaves, a distinguishing feature of the section, are associated with winged cone-scales similar to those of the *Eutacta* group. Two species, both members of the *Colymbea* section, are confined to South America, a continent in which the *Eutacta* section is unrepresented. This section is now confined to Queensland, New Caledonia, Norfolk Island, and the New Hebrides (see Map II).

It has been suggested that the former occurrence of the genus (*Eutacta* section) in the Kerguelen Archipelago is evidence of a southern origin in contrast to the spreading of many conifers from a northern home. *Araucaria* affords a striking instance of the inapplicability of the Age and Area hypothesis to a single genus: its present range is discontinuous and restricted, whereas this ancient member of the Coniferales was formerly almost cosmopolitan.

##### (ii) *The Past Distribution*.

In this short summary of palaeobotanical data the name *Araucaria* is employed in a wide sense as including not only living species, but also extinct forms described by authors either as *Araucaria* or *Araucarites*. Most of the specimens quoted as evidence of the occurrence of *Araucaria* are cones or detached cone-scales: with few exceptions foliage-shoots are not accepted as trustworthy records unless actual evidence of Araucarian affinity is available. Fossil wood of the Araucarian type—that is, characterized by contiguous and polygonal pits on the radial walls of the tracheids—is abundant in rocks of many ages: in itself it is of little value for our present purpose. We cannot as a rule distinguish between *Araucaria* and *Agathis*, nor between the secondary wood of Palaeozoic Cordaitalean and other plants and that of the Araucarineae. We are not concerned with the

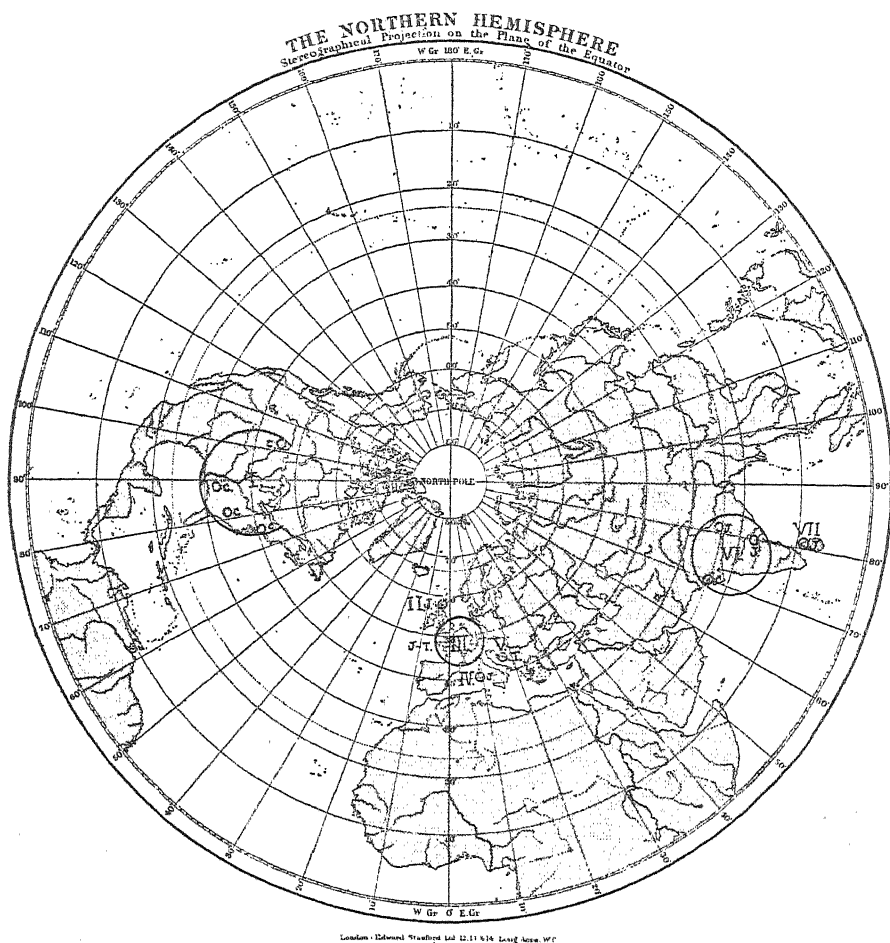


The oldest-recorded occurrence of a single-seeded scale resembling in size and form the cone-scales of recent species is *A. Delafondi* described by Zeiller (87), (Pl. L, Figs. 1, 1 a), from Lower Permian beds (Autunian) near Autun in France. The author of the species, while impressed by the resemblance to *Araucaria*, recognized the possibility of a connexion with *Walchia* or *Ullmannia*. Florin (21) thinks that the Permian fossil 'should probably, as far as can be judged at present, be conceived as belonging to the Araucarineae'. It is, however, by no means certain that the supposed cone-scale is not a winged seed, e.g. a form of the Permian and Carboniferous *Samaropsis* (8). It differs from typical Araucarian scales in the emarginate apex and, though this feature is perhaps of no great importance, the species cannot be accepted as conclusive evidence of the existence of a Permian *Araucaria*. Zeiller (85, Pl. VII, Fig. 6) also described a foliage-shoot from Lower Gondwana (probably Permian) rocks in India as *Araucarites Oldhami* because of a striking resemblance to *Araucaria Bidwillii*. The specimen was re-examined (71, p. 14), and a non-committal name, *Morania* (from the Moran river in India), substituted for *Araucarites* on the ground that Araucarian affinity is by no means certain: subsequently *Moranocladus* was substituted for *Morania* (55, p. 7). Both these late Palaeozoic species—the French and Indian—may be Araucarian, but neither is convincing.

So far as we know, Triassic rocks have not furnished any convincing evidence of the existence of *Araucaria* in the flora of that period, nor has the occurrence of the genus in Rhaetic floras been definitely established. A few years ago numerous silicified cones were discovered in the southern part of the Argentine (territory of Santa Cruz; Map II, 12) which were described by Gothan (26) as *A. Windhausenii* and compared with the Queensland *A. Bidwillii*: Wieland (84), for reasons which are not stated, considered the cone to be intermediate between *Pinus* and *Araucaria*, and substituted the generic name *Proaraucaria*. It is unfortunate that the geological age of these Argentine cones has not been determined: Windhausen speaks of them as Middle Triassic, but Gothan thinks that they may be much younger. The seed-scales as seen in tangential sections of the cone are said to agree with the Colymbea rather than with the Eutacta type: the cone as a whole presents little resemblance to those of the living South American species. Gothan thinks that the cones afford evidence of the existence at some unknown period of a Colymbean *Araucaria* different from the surviving species. Berry (4) described some detached cone-scales, which are almost certainly Araucarian, from a more southern Patagonian locality, as *Araucaria* sp., and compared them with the Indian Jurassic *Araucarites cutchensis* Feist.: he assigns the beds to a Rhaetic age; and Du Toit (75) regards the fossils as indicating Liassic affinities. Another record of an Araucarian cone-scale, which may be Rhaetic, is by Arber (1), who doubtfully assigns a New Zealand specimen of *A. cutchensis*

to the Rhaetic flora of Mt. Potts; the provenance is not definitely known. The same species occurs in Jurassic beds in New Zealand.

One of the oldest examples of an Araucarian cone-scale is the Indian species *A. macropterus* Feist., recently re-described by Sahni (55) : it was



MAP III. Northern Hemisphere, showing regions (larger circles) within which fossil Araucarias have been found : the smaller circles mark localities mentioned in the text. J., C., T.; Jurassic, Cretaceous, Tertiary.

found in the Rajmahal series of the Madras Presidency and in the slightly younger Kota stage. It has been suggested that the Rajmahal series may include beds of Rhaetic or Upper Triassic age, but Sahni (57) thinks that the older estimate of a Jurassic age—not older than Middle Jurassic—is probably correct. It is at least clear that *Araucaria* was widely distributed in the Jurassic period, particularly in the middle and upper stages. Cone-scales such as *A. cutchensis* Feist. (55) have been found in different

parts of India in the Kota and Jabalpur stages; as also in the Lower Cretaceous Umia stage in Kach. This species is recorded by Halle (29) from the Middle Jurassic flora of Graham Land, and whether or not Berry (4) is correct in doubting the specific identity of the Antarctic and Indian specimens, there need be no hesitation in accepting Halle's cone-scales as Araucarian. Many Araucarian cone-scales and cones have been described from Jurassic rocks in England (64, 65); from the Stonesfield Slate (Bathonian), the Inferior Oolite of Yorkshire and elsewhere. Araucarian cone-scales are recorded from Jurassic rocks in France (60) and Germany (17), and recently the Abbé Carpentier (9) has described a species of *Pachyphyllum* as agreeing in cuticular characters with living species of *Araucaria*. A Jurassic cone-scale has also been found in Sardinia (38). Cone-scales similar to those from Europe and India are recorded from Jurassic rocks in Victoria and New South Wales (66, 79, 80); and, as already stated, in New Zealand.

Araucarian cones and cone-scales occur in Lower Cretaceous strata (Wealden) of England and France. A species, *Araucaria bohémica* Vel. (77), from higher Cretaceous beds in Bohemia, though possibly Araucarian, is not convincing. Foliage-shoots found in a boring through Cretaceous rocks in Holland are assigned by Kräusel (39) to *Araucaria* because of a resemblance in cuticular structure to *A. Bidwillii*. An Italian Cretaceous form, rashly named *A. macrophylla* Bozzi (5), is characterized by large flat leaves, and, as the author of the species says, it resembles the *Colymbea* section of the genus: it is similar to the North American species *Araucarites ovatus* Holl.; but we know nothing of the structure of the leaves.

The oldest Araucarian fossil from North America is a species, *A. wyomingensis* Font. (81), founded on cone-scales from the Lower Cretaceous formation of the Black Hills of Dakota. Another species, *Araucaria Jeffreysi* Berry (2), represented by cone-scales, is recorded from higher Cretaceous beds in North Carolina; associated with the scales are impressions of foliage-shoots very similar to those of *A. Bidwillii*. The flat-leaved shoots, though possibly belonging to the tree from which the cone-scales were derived, are described as *A. bladenensis* (3); this species has been found in North and South Carolina, in Georgia, and in Alabama in Middle and Upper Cretaceous beds; it closely resembles a French Cretaceous form, *A. Toucasi* Sap. (59, p. 198, Fig. 27), from Cenomanian strata in southern France. A similar type of foliage-shoot with rather larger and less pointed leaves is *A. ovatus* (32) from Cretaceous beds in New Jersey. Wieland (83) has given a brief and unconvincing description of two Upper Cretaceous species from Wyoming and South Dakota which he speaks of as two new Araucarias: one is founded on a cone and the other on a piece of stem with leaves. They may be Araucarian. The occurrence of *Araucaria* in the early Cretaceous vegetation of Australia (78, 79) is shown by

cone-scales (*Araucarites Arberi* Walk.) from the Burrum Series of Queensland. *A. mesozoica* Walk. from the Cretaceous Maryborough Series, founded on leaves, is of little value as a record of *Araucaria*, and the same may be said of *Araucarites* (?) *polycarpa* Ten. Woods. From Senonian beds in New Zealand Edwards (19) has described a foliage shoot as *A. Marshalli*, which he compares with the American *A. ovatus* Holl. and other species: the specimens are, no doubt, correctly regarded as Araucarian. He also describes some wood believed to be Araucarian as *Dadoxylon kaiparens*.

Evidence of the existence of *Araucaria* in early Tertiary floras of Europe is derived from vegetative shoots, some of which have been shown to agree in cuticular characters with recent species. The species known as *Araucarites Goepperti* (Sternb.) is represented by good specimens in the Eocene beds of Bournemouth (23), also in Lower Tertiary beds in the Island of Mull, the Tyrol, France, Switzerland, Italy, Dalmatia, and elsewhere. Miss Bandulska, who made a careful examination of cuticles of the Bournemouth material, regards *A. Goepperti* as nearest to *Araucaria excelsa*. A very similar form of foliage-shoot from Oligocene beds in the Isle of Wight has been named by Florin *Araucarites Gurnardi* (50); he thinks that the flora contained more than one species. The stomata agree very well on the whole with those of *Araucaria*: Florin also describes the cuticular structure of the allied and widely spread *Araucarites Goepperti* as remarkably uniform. Cones and cone-scales, probably Araucarian, and in some localities associated with Araucarian foliage-shoots, are described by several authors under the generic name *Doliostrobus*: they are probably Araucarian.

We are not aware of any satisfactory evidence of the occurrence of *Araucaria* in Tertiary floras of North America. From Tertiary beds, probably Oligocene, in the Magellan Straits region, Dusén (15) has described foliage-shoots as a new species, *A. Nathorsti*, which recalls *A. ovatus* Holl., a New Jersey form. The same author records another species—also founded on leaves—from early Tertiary beds on Seymour Island (16) as *A. imponens*, resembling *A. brasiliensis* and *A. Bidwillii*. From Seymour Island and Snow Hill Gothan (25) described petrified wood, which may well be Araucarian, as *Dadoxylon* (*Araucarioxylon*) *pseudoparenchymatosum*. A branch from Macquarie Harbour, Tasmania, figured by Johnston as *Araucaria imbricatiformis* (36, Pl. 36, Fig. 1), is similar to the Italian species *A. macrophylla* Bozzi, and, though not improbably Araucarian, cannot be accepted as a satisfactory record.

So far we have given a general review of such fossils as seems to us of value as records of Araucarian species, and before we venture to draw any conclusions from the evidence it is worth while to draw attention to the lack of satisfactory records in certain parts of the world. It is significant that there appears to be no convincing evidence of the occurrence of

*Araucaria* in pre-Jurassic, Jurassic, Cretaceous, or Tertiary floras in the Arctic regions. The foliage-shoots described by Nathorst (46) from early Cretaceous or later Jurassic rocks in Spitsbergen as *Elatides curvifolius* (Dunk), a Wealden and late Jurassic European species, may be Araucarian, but no actual proof has so far been obtained. Some small cone-scales from the early Cretaceous flora of western Greenland referred to the genus *Protodammara* (68, Pl. 10, Figs. 90, 94), though possibly Araucarian, cannot safely be accepted as definite evidence. One cannot help being struck by the relative lack of Arctic foliage-shoots and cones suggestive of Araucarian affinity in contrast to the wide distribution of other Conifers closely related to the existing species of *Sequoia*, Abietineae, and other families. Cretaceous and Tertiary wood of the *Araucarioxylon* type occurs in early Cretaceous localities in the Libyan desert. A cone, *Conites araucarioides* Goth. (27), from Upper Jurassic or Lower Cretaceous of East Africa, regarded by Gothan as Araucarian, does not afford convincing evidence of affinity.

We have searched in vain for Araucarian fossils in the accounts of fossil floras from Siberia, China, and Japan. Schenk (62) described a fragment of foliage-shoot from Jurassic rocks in China as *Araucaria prodromus*, but the specimen affords no clue to systematic position. From the rich Rhaetic flora of Tonkin, Zeiller (86) described a cone as *Triolepis Leclerii*, which he compared with *Cunninghamia*, and as that genus was formerly included in the Araucarineae he regarded the fossil as possibly Araucarian: the scales bear three seeds.

We know very little of the fossil floras of New Caledonia, an island which has a strong claim to be regarded as the present headquarters of *Araucaria*. Crié (12) described some wood—*Araucarioxylon australe*—from beds said to be Triassic, but that in itself is of little importance.

On the map of the northern hemisphere three separate areas are shown within which Araucarian remains have been found: from American localities only Cretaceous species; from India, Jurassic and Lower Cretaceous; from Europe, Jurassic, Cretaceous, and Tertiary. Plant-bearing Jurassic rocks are very poorly represented in North America, and occur only in Oregon: on the other hand, there are many rich Tertiary floras, and the lack of *Araucaria* is probably not a result of the imperfection of the geological record. There are many localities in Asia where Jurassic plants are abundant; but from none have undoubted Araucarian fossils been obtained. It would seem improbable that *Araucaria* came originally from the far north and spread along divergent routes towards the equator: one would expect to find traces of its path over Siberia and China and in regions more northerly than those shown on the map in America and Europe.

## PHYTOGEOGRAPHICAL AND PALAEOGEOGRAPHICAL PROBLEMS.

Sir Joseph Hooker was driven to the conclusion that the Archipelago is a remnant of a much larger land-area which extended near enough to South America, either as a continuous continent or a series of island stepping-stones, to receive immigrants from Fuegia. Darwin, in the 'Origin of Species', suggested the possibility of seed transport over a wide expanse of sea by floating ice; but this, it is generally agreed, cannot be admitted. He believed that there must have existed a Tertiary Antarctic continent from which various forms radiated to the southern extremities of our present continents. W. R. B. Oliver (48), on the other hand, is opposed to the idea of foundered continents—'the easy method by which these hypothetical continents can be brought up from the depths of the ocean has probably been one cause why the study of geographical distribution has made little advance in recent years.' Cockayne (10) and Skottsberg both visualize an Antarctic continent as a centre of evolution. Sir Arthur Hill (31) is another advocate of an Antarctic origin: he speaks of the former existence of an extensive land-area—Antarctica—which enjoyed from time to time 'a temperate or even a subtropical climate'. An extension of Antarctica may be conceded; but a subtropical climate is an assumption much more difficult to defend. In creating climates to suit the apparent requirements of an extinct flora, such as that which has left abundant samples in the Jurassic rocks of Graham Land (29), we are confronted with meteorological obstacles which almost demand acceptance, in some form, of Wegener's conception of a mobile crust. Raising foundered continents from ocean depths may be defended on the ground that in the Andes and elsewhere there is abundant evidence of vertical movement through several thousand feet in the course of the Tertiary period; but the problem is to make the raised continental blocks and bridges fit the climatic conditions which seem to be demanded by the relics of an earlier vegetation. The former existence of *Araucaria* (confirmed by the fossils described in this paper) in the Archipelago would seem to demand either a land-bridge or the adoption of some form of continental drift. Whether or not we are disposed to admit the possibility of the introduction by natural causes of mosses, ferns, and flowering plants across many hundred of miles of ocean by wind, birds, or currents, it is inconceivable that *Araucaria* could by any means have accomplished so long an ocean journey. Dr. Guppy (28) states that he never found seeds of the Araucarian genus *Agathis* in floating drift or on sea beaches; he found that fresh cones sank and seed-scales floated only a few hours. The winged seed-scales might be scattered locally by wind; they are unsuited to transport by birds. In this connexion it is worth noting that Professor Compton (11), in his account of New Caledonian plants, states that the



whole conifer flora is endemic, and he attributes this to the imperfection of their means of seed-dispersal. The presence of *Araucaria* is evidence of climatic conditions more genial than the present. We know that some species are tolerant of relatively cold situations; *Araucaria* (70) grows at a height of 3,000 feet in the forests of Brazil, it overtops other trees on the summits of New Caledonian mountains 3,500 feet above sea-level, and occurs at altitudes of 6,000 feet and upwards in New Guinea. *Araucaria araucana* Mol. (= *A. imbricata* Pav.) is said to be cultivated as far north as 62° 41' in Norway. While it is inconceivable that the genus could exist to-day on the Kerguelen Archipelago, no very great change in physical conditions need be postulated. Dr. Ridley (51) considers that the seeds of several Kerguelen species could be carried long distances in mud on the feet of birds or on their feathers: he quotes the wingless flies of Kerguelen and some other sub-antarctic islands, also species of freshwater fish<sup>1</sup> common to Kerguelen, New Zealand, Tasmania, and South America as evidence of a former continent. There are also certain earthworms and other animals which were common to the Archipelago and New Zealand.

Unless we revert to the pre-Darwinian supposition of multiple centres of creation, or stretch beyond reasonable limits our faith in the efficiency of dispersal agencies, we cannot believe that the Kerguelen Archipelago and other islands in the southern ocean occupied their present positions in relation to the continents when they were first colonized by the conifers of a former age, or even by the ancestors of the present flowering plants.

The origin of the present flora, it has been urged, must have been post-glacial (7), that is subsequent to the more extensive glaciation of the Archipelago of which there is abundant evidence. It is implied that the pre-glacial vegetation was destroyed during the Quaternary Ice Age: though possibly some species were unable to endure the severe climate, it is unlikely that destruction was complete. Heard Island is now in much the same state of glaciation as Kerguelen Land was, and yet several vascular plants are able to exist. Recent research in the northern hemisphere tends to show that most estimates of loss during the last Ice Age are excessive. If the flora has received post-glacial additions which are not endemic species they must have crossed wide seas from a western or an eastern home. Reference has already been made to the possibility of seed-dispersal by birds and to the great demands such method of transport makes upon our belief in the efficiency of this method for distances of 1,000 miles and more. We know that wind is a potent factor in the dispersal of spores and light fruits and seeds—and in this connexion it is relevant to quote from Professor Rudmose Brown's paper the occurrence of pollen of an Andean *Podocarpus* in red snow on the south Orkneys—

<sup>1</sup> The supposed freshwater fish (a species of *Galaxias*) is now known to breed in the sea: see (45), p. 357.

but, so far as we are aware, there are no authentic records of the germination of wind-borne seeds which have travelled by ocean routes over distances comparable with those required by the remoteness of the Kerguelen Archipelago.

The contribution made by Dr. de la Rüe to the botanical history of Kerguelen Land recalls a passage in a letter written in 1875 by Darwin to Oswald Heer, who had recently described Arctic fossil floras: he wrote—‘How I wish that similar collections could be made in the southern hemisphere, for instance, in Kerguelen’s Land’ (13, Vol. II, p. 240). This wish has been fulfilled, not only through the fossils from Kerguelen Land, but by the work of the Swedish Expedition to Graham Land and the discovery of *Glossopteris* on the Antarctic continent (20; see also Map II, B., F., P.). Hooker wrote to Oliver ‘perhaps the fossil wood of Kerguelen’s Land may be the nucleus of a great light’ (35, Vol. II, p. 203): one is tempted to prophecy that discoveries in the future will enable us with more knowledge and less speculation to reconstruct the part played by an Antarctica of past ages as a centre of evolution.

How then, are we to account for the present flora of Kerguelen Land and the flora of a pre-glacial age? There is no evidence of any substantial change in area or height above sea-level of the Archipelago since the retreat of the ice. It would seem impossible for the sub-antarctic islands to be colonized by sea-borne or air-borne seeds unless they were much more favourably placed than at present in relation to the sources of supply. When we consider *Araucaria* and other members of the extinct flora the case for a lost land-bridge is enormously strengthened. The Kerguelen Archipelago must have been part of a continent: whether the parent mass lies below the southern ocean, or whether the submarine platform with its covering of basalt-sheets is a detached portion which floated away from a continental block is a question to which as yet no definite answer can be given. Attention may be called to an interesting paper by Brockmann-Jerosch (6) on the south polar tree-limit, which deals with questions relevant to the flora of Kerguelen and other sub-antarctic islands.

We are not exclusively concerned with possibilities of plant-dispersal and the stocking of areas that are now oceanic islands; there is also a climatic problem. Phytogeography is not only intimately connected with geology; it may receive substantial assistance from geophysics. Improved methods of sounding have shown that some parts of the ocean-floor are counterparts of mountainous regions of continents rather than submarine plains: former isthmian links from continent to continent, as recently suggested by two American geologists (63), may be imagined with reasonable justification, but it is very doubtful if they in themselves will supply adequate explanations of climatic conditions necessitated by extinct floras. Vertical movements alone are insufficient; lateral displacement of conti-

nents seems to be an almost necessary assumption. It is stated that recent soundings reveal contours on the sea-floor comparable with those of continents (52); and that the South Antillean arc, geologically and geographically, shows a mixture of oceanic and continental characters. These and other arguments have been advanced against the acceptance of Wegener's theory with its 'absurd phytogeographical consequences', which du Rietz considers are demonstrated by Diels and other authors whom he quotes. Though it may be conceded that the difficulties presented by the past and present distribution of plants cannot be satisfactorily disposed of by the acceptance of Wegener's theory as he propounded it, this concession does not preclude belief in some form of continental drift.

The Wegener hypothesis, despite the serious criticism which it has raised, appeals strongly to the imagination. There are other conceptions of a drifting sial, such, for example, as the bold hypothesis proposed by Gutenberg (40). It is by no means improbable that solutions of some of the many problems of Plant Geography—both past and present—will be found, not in the raising of foundered continents, but through the acceptance of the mobility of the earth's crust, as a factor not merely imagined but substantiated by evidence which, it may be suggested, will eventually be provided.

#### SUMMARY.

The discovery by Dr. de la Rüe of fossil plants in beds associated with the volcanic rocks of the Kerguelen Archipelago afforded an opportunity of considering the various problems connected with the botanical history of the sub-antarctic islands. A general account is given of the geology of the Kerguelen Archipelago based on the work of Dr. de la Rüe and previous explorers, also of the main features and geographical relationships of the present flora.

The fossil plants are described. Their geological age is believed to be Tertiary, though it is not considered possible to date them within narrower limits. Special attention is paid to the geographical range of present and past representatives of the genus *Araucaria*.

The paper concludes with a review of phytogeographical and palaeogeographical problems with special reference to the Kerguelen Archipelago.

#### POSTSCRIPT.

I am greatly indebted to Mr. Stanley Kemp for allowing me to see a sketch-chart of soundings taken by *Discovery II* and sketched in the course of her circumpolar cruise. These soundings afford confirmatory evidence of a connecting ridge between the Kerguelen Archipelago and Kaiser Wilhelm II Land, on which Gaussberg is situated. The *Discovery* soundings had not been plotted when this paper was written.

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## EXPLANATION OF PLATES XIV AND XV.

Illustrating Professor A. C. Seward and Miss Verona Conway's paper on 'A Phytogeographical Problem : Fossil Plants from the Kerguelen Archipelago'.

### PLATE XIV.

Figs. 2-15. E. T. Scott phot.

- Fig. 1. A scene in the Kerguelen Archipelago. Dr. de la Rue phot.
- Fig. 2. *Araucarites Ruei* sp. nov. Nat. size.
- Fig. 3. *Araucarites Ruei* sp. nov.  $\times 1\frac{1}{4}$ .
- Fig. 4. *Araucarites Ruei* sp. nov.  $\times 1\frac{1}{4}$ .
- Fig. 5. *Elatocladus kerguelensis* sp. nov.  $\times 2\frac{1}{2}$ .
- Fig. 6. *Filicites* sp. A.  $\times 2\frac{1}{4}$ .
- Fig. 7. *Desmiophyllum* sp.  $\times 1\frac{1}{2}$ .
- Fig. 8. *Araucarites Ruei*. Nat. size.
- Fig. 9. *Araucarites Ruei*.  $\times 2$ .
- Fig. 10. *Phyllites kerguelensis* sp. nov.  $\times 1\frac{1}{5}$ .
- Fig. 11. *Elatocladus* sp. circa. nat. size.
- Fig. 12. *Dicotylophyllum Edwardsi* sp. nov.  $\times 1\frac{3}{5}$ .
- Fig. 13. *Araucarites Ruei* (cone-scales).  $\times 3$ .
- Fig. 14. *Araucarites Ruei* (cone-scales). Nat. size.
- Fig. 15. *Dicotylophyllum* sp. B.  $\times 4$ .

PLATE XV.

All figs. E. T. Scott phot.

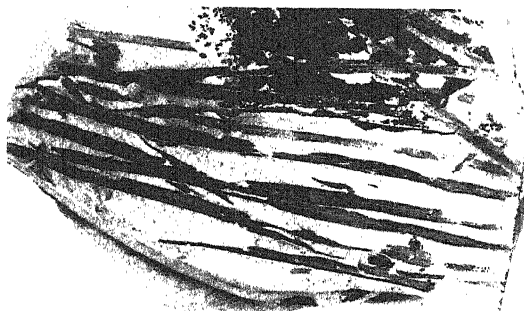
- Figs. 16, 16 a. *Desmiophyllum* sp. Fig. 16 nat. size.  
 Fig. 17. *Desmiophyllum* sp.  $\times 2$ .  
 Fig. 18. *Elatocladus kerguelensis*.  $\times 2$ .  
 Fig. 18 a. *Elatocladus kerguelensis*.  $\times 2\frac{1}{2}$ .  
 Fig. 19. *Dicotylophyllum* sp. Nat. size.  
 Fig. 20. *Araucarites Ruei*. Nat. size.  
 Fig. 21. *Dicotylophyllum* sp. Nat. size.  
 Fig. 22. *Dicotylophyllum Edwardsi*.  $\times 1\frac{1}{4}$ .  
 Fig. 23. *Muscites* sp. (? *Dicranites australis*).  $\times 2\frac{1}{2}$ .  
 Fig. 24. *Dicotylophyllum* sp.  $\times 2\frac{3}{4}$ .  
 Fig. 25. *Araucarites Ruei*.  $\times 1\frac{1}{2}$ .  
 Fig. 26. *Desmiophyllum* sp. Nat. size.  
 Fig. 27. Part of a young fern.  $\times 3\frac{1}{2}$ .  
 Fig. 28. *Dicranites australis* Dix.  $\times 2\frac{1}{2}$ .  
 Fig. 29. *Filicites* sp. A.  $\times 1\frac{3}{4}$ .  
 Fig. 30. *Filicites* sp. A.  $\times 2$ .  
 Fig. 31. *Filicites* sp. B.  $\times 2\frac{1}{2}$ .  
 Fig. 32. *Filicites* sp. B.  $\times 2\frac{1}{4}$ .  
 Fig. 33. *Filicites* sp. circa. nat. size.



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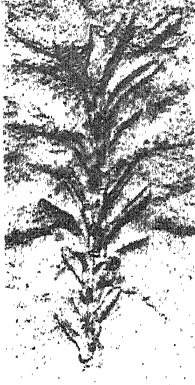




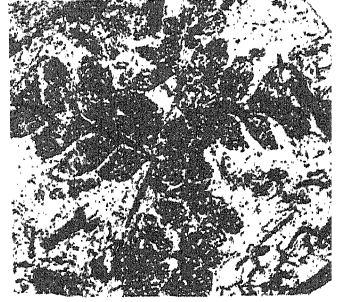
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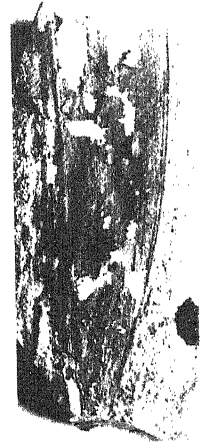
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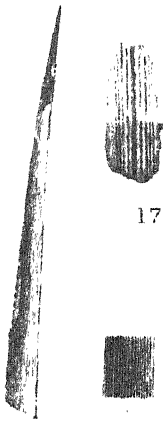
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# Studies in the Absorption of Calcium from Nutrient Solutions with Special Reference to the Presence or Absence of Boron.

BY

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With eight Figures in the Text.

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## I. INTRODUCTION.

SOME investigations into the role of boron in plant growth which were the subject of a previous paper (1), gave rise to the suggestion that a definite association existed between this element and the absorption or utilization of calcium. This conclusion seemed warranted as, judging from the dry weight produced, *Vicia faba* plants appeared able to make better use of deficient quantities of calcium if boron were also supplied. In contrast to what obtained when other nutrient elements were lacking, death from a shortage of calcium followed more rapidly in the absence than in the presence of boron, and further, symptoms of a deficiency of calcium showed a close resemblance to those of a lack of boron in that both affected the apical portions of the shoot first, and caused a somewhat similar discoloration and withering. Both Reed and Haas (31) and Nightingale et al. (25), working with young orange trees and tomatoes respectively, also describe apical death as characteristic of a shortage of calcium, while McMurtrey (22) shows how the effects of a deficiency of calcium and boron may be distinguished in the case of tobacco. In the case of a deficiency of any of the other essential elements the appropriate symptoms either made their first appearance in the lower parts of the plant or were in other ways readily distinguished from those due to a deficiency of boron. No actual analyses were carried out in these experiments, and it seemed desirable to make a further study of this possible calcium-boron association. Quantitative estimations were therefore carried out to determine the amount of calcium absorbed by the plant at different stages of growth and under varying nutritional conditions, both in the presence and absence of boron. Water-culture methods were used throughout, as by this means the quantity of calcium supplied could be most readily controlled and the absorption most accurately measured.

The hope of obtaining a clearly defined answer to the problem was, however, not realized, but results of considerable interest were secured which are presented in the following paper.

## A. GENERAL OUTLINE OF THE EXPERIMENTS.

A modification of the usual Rothamsted solution<sup>1</sup> was used, calcium

<sup>1</sup> KNO<sub>3</sub> 1.0 grm. KH<sub>2</sub>PO<sub>4</sub> 0.3 grm. K<sub>2</sub>HPO<sub>4</sub> 0.27 grm. MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5 grm. CaCl<sub>2</sub> as required. FeCl<sub>3</sub> 0.04 grm. Distilled water 1,000 c.c.

chloride being substituted for the sulphate so as to obtain more accurate measurement of the calcium supplied, and sodium chloride being omitted in order to avoid excess chlorine ions. This solution was made up in bulk *without* the addition of the calcium or ferric salts and the culture bottles (capacity 600 c.c.) filled. Calcium chloride, of which a standardized stock had been prepared, was then added with a pipette as desired, followed by a uniform quantity of ferric chloride solution to each bottle. The pH value of the solution was 6.2, and no alteration in this figure was obtained when the concentration of calcium was reduced. Single plants only were set up in each bottle and excellent growth was made considering the comparatively late season of the year (September–October or November) at which the experiments were carried out. No artificial light was employed.

Since *V. faba* had been chiefly grown in the earlier work, the same plant and variety (Sutton's Prolific Longpod) was used in the present experiments. The seed was graded by weight, but as such a large number of plants was required a somewhat wide range was unavoidable. Care, however, was taken to ensure random distribution of the seeds throughout the different treatments.

In the first season (1930) the nutrient solution supplied contained 52.49 mg. calcium per plant, and special attention was paid to the amount of this element absorbed at different stages of growth when the nutrient medium was both unchanged or renewed at regular intervals. A parallel study of absorption from a pure solution of calcium chloride was carried out at the same time. Each of the treatments was repeated with the addition of 1 p.p.m. boric acid, five or ten plants serving as the unit. The treatments may be summarized as follows:

	Treatment.	Duration.
(i) Nutrient solution.	Renewed weekly after first 2 weeks <sup>1</sup>	9 weeks
(ii) " "	" " " 3 "	9 "
(iii) " "	Renewed fortnightly after first 3 weeks	9 "
(iv) " "	Unrenewed	2-5 "
(v) Calcium chloride solution alone	Unrenewed	4 "

In the following year (1931) the influence of the quantity of calcium supplied on the amount of calcium absorbed by the plant was the chief question studied. The same basal culture solution was used as in the previous season, but the calcium chloride content was varied so as to supply one of the following amounts of calcium:

54.365	mg. calcium per plant (afterwards referred to as full quantity)				
27.1825	" "	" "	" "	$\frac{1}{2}$	"
13.5913	" "	" "	" "	$\frac{1}{4}$	"
6.7957	" "	" "	" "	$\frac{1}{8}$	"

<sup>1</sup> Experience had shown that three weeks was a most satisfactory interval to allow before giving the first renewal of solution, but this additional series was included in case appreciable absorption of calcium had occurred during the first fortnight.

As before, the whole series was repeated with the addition of 1 p.p.m. boric acid. The plants were carried on for five weeks only, as, except for the occasional addition of a trace of ferric chloride, no renewal of the solutions was made.

Growth was rather less vigorous than in the previous year, as the dry-weight figures show (Table II), but the plants were entirely healthy and had nearly reached the flowering stage at the end of the five weeks' growth. Although direct comparison between the performance of the plants in the two seasons cannot be made owing to the inevitable differences in external conditions (which would necessarily affect the rates of growth and absorption) and also to the slightly larger amount of calcium supplied as the 'full' quantity in the second season, yet the results from the two-years' investigations, where repeated, are consistent (Table II), so that the additional support is afforded to the accuracy of those other parts of the experiment for which no repetition was possible.

#### B. ANALYTICAL PROCEDURE.

The used solutions were reserved for analysis every time that plants were harvested or were transferred to a fresh bottle of solution for further growth. As it was of equal importance that no calcium, whether as solution or precipitate, should be left adhering to the plants in either case, the roots were rinsed with a 2 per cent. solution of HCl, followed by distilled water and the washings added to the appropriate solution before analysis. No apparent damage to later growth resulted from this treatment. Two cubic centimetres of concentrated HCl were then added to each bottle preparatory to analysis, in order to ensure the complete solution of all the calcium present, and to prevent the growth of fungi and algae during the interval before the estimations were made.

As most of the concentrations were too weak to ensure sufficient calcium being left in a single bottle for accurate estimation, the solutions from two, three, or five plants were bulked before analysis in the case of the one-half, one-quarter, and one-eighth doses of calcium respectively, triplicate determinations being carried out. Separate estimations, however, were made on the solutions from plants receiving the full supply of calcium, five replicates being available in this case.

After evaporation to a convenient volume the calcium was precipitated as oxalate and estimated by titration with standard potassium permanganate after Chapman's method (4). With the exception of the solution containing the largest concentration of calcium chloride, the high proportion of magnesium to calcium offered some difficulty, as magnesium oxalate tended to be precipitated at the same time as the calcium salt. This trouble was satisfactorily overcome by dissolving the mixture of



magnesium and calcium oxalates in hot HCl and repeating the precipitation as before, the calcium oxalate then being obtained pure.

As a further check on the methods employed, estimations were carried out on the plant ash to see if the loss of calcium from the nutrient medium could be accounted for by that found in the plant itself. The results are given in Table I. Allowance for the initial calcium content of the seed must, of course, be made before comparing the two sets of calcium values. The variation between the dry weights of individual seeds was large, so that the figure for the calcium content per seed is necessarily a mean value only, but taking this into consideration the agreement would seem to be satisfactory.

TABLE I.

*Comparison between the Calcium in the Plant Ash and that Lost from the Nutrient Solution after Four Weeks' Growth.*

	(mg. per plant. Average of 5.)		Ca Uptake. <sup>1</sup>	
	Seed.	Whole plant.	As found in plant less seed.	As lost from solution.
+ B	2.0	16.7	14.7	15.1
No B	2.0	9.6	7.6	7.1

## II. EXPERIMENTAL DATA.

### A. CALCIUM ABSORBED AT DIFFERENT STAGES OF GROWTH FROM A COMPLETE NUTRIENT SOLUTION.

#### (a) *Boron supplied.*

##### (1) Actual uptake of calcium.

In the case of plants whose culture solution was renewed regularly every week from the end of the second week, and which, therefore, always had access to an ample supply of nutrients, the calcium absorbed increased (with a single exception) up to the seventh week, remained approximately at the same level for a further seven days and then fell significantly during the last, i.e. the ninth week (Fig. 1). By this time the plants were well grown and in flower, but light conditions were becoming unsuitable for vigorous growth, so the experiment was discontinued and data for more mature plants are not available. Burd (3), working with barley, records a similar decrease in the rate of absorption of nutrients after the ninth week of growth, actual loss occurring in the case of calcium. Confirmation of the seventh week as the period of maximum calcium absorption under

<sup>1</sup> In the case of the solutions the average is the mean of 5 separate determinations, but the values for the ash analyses are the means of duplicate determinations on material bulked from 5 plants.

these particular experimental conditions was provided by five other plants grown simultaneously, whose treatment was identical except for the fact that the weekly renewal of their solution was not begun until the third

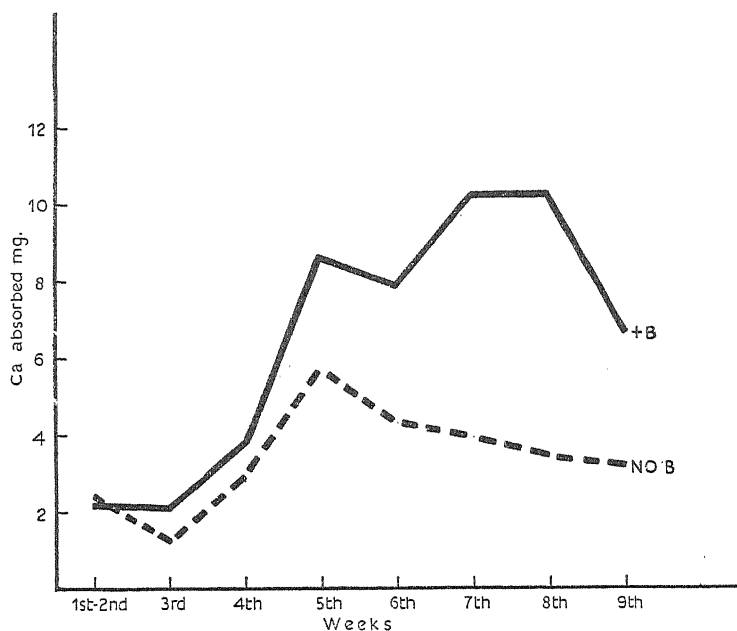


FIG. 1. Calcium absorbed by *Vicia faba* in successive weeks from a complete nutrient solution renewed weekly. 1930.

instead of the second week. A slight check in the uptake of calcium occurred in both sets of plants during the sixth week, and although a statistical examination of the figures showed that any actual decrease lies within experimental error, yet it is evident that at this period the rate of increase in the absorption of calcium was less vigorous than during either the preceding or following week. Calcium uptake would, therefore, seem to be periodic in nature, as has been generally found by a number of workers with other elements and plants (see Lundegårdh (21)). The failure of Redfern (30) to obtain evidence for periodicity in uptake is probably to be accounted for by the short period (36–84 hours) for which her experiments were continued. Fonder's work (8) perhaps offers the closest comparison with the present results, as he also used the bean plant. With a variety of soil types he found a reduction in percentage of calcium in the shoot at budding or fruiting time, which was followed by a rise when complete maturity was attained. It is, therefore, possible that in the present case this final rise was missed owing to the plants being harvested when flowering was reached.

Where the culture solution was renewed every fortnight, after remaining unchanged for the first three weeks, the general course of the curve closely resembled that where fresh solution was supplied weekly, viz. the maximum

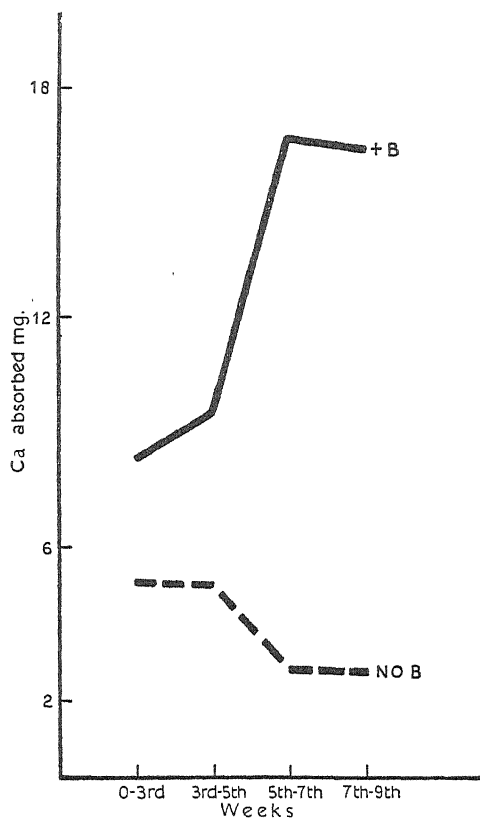


FIG. 2. Calcium absorbed by *V. faba* during successive growth periods from a complete nutrient solution renewed fortnightly. 1930.

uptake of calcium was reached by the seventh week, and although no definite decrease took place during the following two-week period, at least no further rise occurred (Fig. 2).

In the case of plants whose solutions were not renewed at all, figures are available for the first five weeks only, as the general nutrient deficiency which sets in about this date would have inevitably vitiated the results if the plants had been grown on longer. In the 1930 unrenewed series a steady increase in the quantity of calcium absorbed took place with each additional week for which the plants were grown (Fig. 3), and, further, the amount taken up per week also increased up to the end of the experiment (Table II). When this series was repeated in the following year, a drop in the uptake of calcium occurred during the fifth week, so that it seems

probable that the figure for the final week in the previous season had been a maximum point. In this connexion it is interesting to note that the actual amount of calcium absorbed during this probable peak period

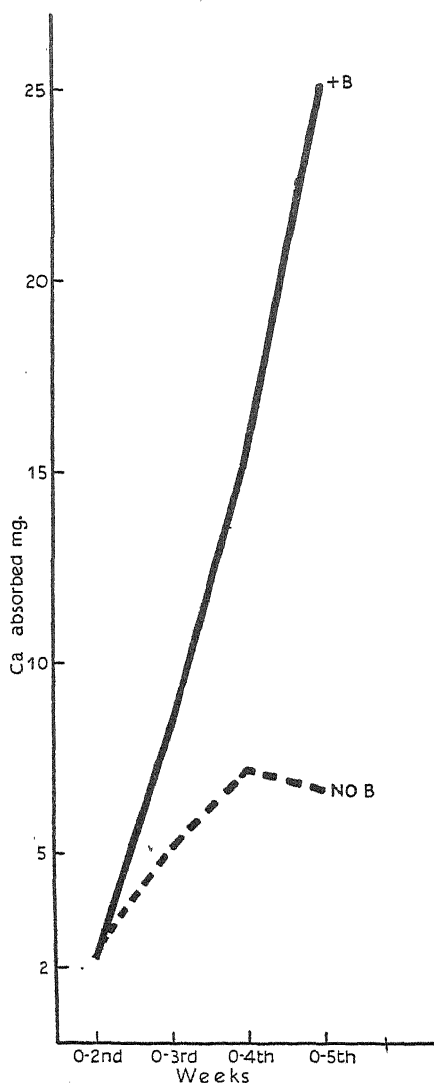


FIG. 3. Calcium absorbed by *V. faba* during progressively increasing periods of growth from a complete nutrient solution not renewed. 1930.

(10 mg. per plant) was practically identical with that taken up by plants receiving weekly renewed solutions during *their* week of maximum absorption (Fig. 1), although in the latter instance the highest point was not reached until a fortnight later.

Many factors can account for the more rapid attainment of the peak period of absorption by the plants in unrenewed solutions, the most important of which is probably the absence of the disturbing factor of the actual renewal itself, especially in the early stages of growth, as will be seen in a later section. The greater reduction in the concentration of all nutrients, the increased variation in balance and pH value of the unchanged medium compared with that which was frequently renewed, would also account for differences in the quantity of calcium absorbed, and the falling off in calcium uptake in the fifth week of the unrenewed series in 1931 is probably to be attributed to these or seasonal factors rather than to any shortage of calcium itself. In support of this, only 25.3 per cent. of the total calcium supplied had been taken up by the end of the fourth week when the absorption began to decrease, although it is of course possible that such factors as are referred to above, increase in alkalinity, changes in balance, concentration, &c., might have induced a deficiency of *available* calcium as distinct from total calcium.

(2) Uptake of calcium per cent. dry matter.

In order to eliminate the effect of individual variation in size and thereby to afford a stricter comparison between the performance of plants receiving different treatments, the calcium absorbed has, where possible, been reckoned as a percentage of the dry matter produced (Table II). Calculated on this basis, the uptake of calcium in the unrenewed series followed the same course as the actual calcium absorbed, i.e. increased with every additional week of the five-week period for which the plants were grown in the 1930 series, no further rise occurring after the fourth week in the repetition experiment in the succeeding year.

TABLE II.

*Calcium Absorbed from a Complete Nutrient Solution (with Boron) and Dry Weight Produced at Different Stages of Growth.*

Average of 5 plants.			(Solution not renewed.)		
Age weeks.	Actual Ca absorbed.		Dry weight.	Ca absorbed per cent. dry weight.	
	Total.	Weekly increment.			
mg.			gram.		
1930 {	2	2.2	2.2	1.43	0.154
	3	8.3	6.1	2.09	0.397
	4	15.1	6.8	3.15	0.479
	5	25.1	10.0	4.35	0.577
1931 {	2	2.2	2.2	1.15	0.188
	3	6.2	4.0	1.57	0.395
	4	13.8	7.6	2.42	0.570
	5	17.1	3.3	2.39	0.504
3 D					

Figures for more mature plants are afforded by the series whose solutions were renewed fortnightly or weekly. In the former case, the calcium absorbed per cent. of dry weight produced during the sixth to ninth weeks showed a considerable advance on that taken up by the same plants during the previous five-week period (Table III). The results from the weekly renewed series (Table IV), on the other hand, are at first sight contradictory, as the calcium absorbed per cent. of dry weight after nine weeks is very little, if at all, larger than that absorbed in the first five weeks by plants in either unrenewed or fortnightly renewed solutions (Tables II and III). Closer inspection, however, reveals the fact that the *average* value for the complete nine weeks in the fortnightly renewed series is practically identical with that in the weekly renewed set during the same period, showing that the fluctuation in uptake had been lost sight of when the final value only was available. Intermediate figures, however, were unable to be obtained as it was impossible to determine dry weights at successive stages when identical plants were under observation for a series of weeks.

TABLE III.

*Calcium Absorbed from a Complete Nutrient Solution and Dry Matter Produced at Different Stages of Growth.*

Average of 5 plants.		(Solutions renewed fortnightly.)			Average.
		Total Ca absorbed. mg.	Dry weight. gram.	Ca absorbed per cent. dry weight.	
+ B	{ 1st-5th week	17.8	4.36	0.408	} 0.584
	{ 6th-9th week	33.1	4.36	0.759	
No B	{ 1st-5th week	10.1	2.80	0.396	} 0.379
	{ 6th-9th week	5.5	1.39	0.396	

TABLE IV.

*Calcium Absorbed from a Complete Nutrient Solution and Dry Matter Produced after Nine Weeks' Growth.*

Average of 10 plants.		(Solutions renewed weekly.)		
		Total Ca absorbed. mg.	Dry weight. gram.	Ca absorbed per cent. dry weight.
+ B		54.83	9.22	0.588
No B		26.65	4.84	0.548

(b) *No boron.*

(1) Actual uptake of calcium.

A lack of boron brought about distinct differences in the uptake of calcium. In the first place the amount of calcium absorbed increased with

age up to the fourth or fifth week only, irrespective of the frequency with which the culture solution was renewed, and further, the actual quantity absorbed each week was smaller and the rate of increase in the uptake slower than in the case of plants supplied with boron (Figs. 1, 2, and 3 and Table V). Where it was possible to grow the cultures on for nine weeks (weekly and fortnightly renewed series) the quantity of calcium taken up steadily decreased after the fourth or fifth week until the end of the experiment.

TABLE V.

*Calcium Absorbed from a Complete Nutrient Solution (without Boron) and Dry Weight Produced at Different Stages of Growth.*

Average of 5 plants.				(Solution not renewed.)	
Age weeks.	Actual Ca absorbed.		Dry weight.	Ca absorbed	
	Total.	Weekly increment.		per cent. dry weight.	gram.
		mg.			
1930 {	2	2.6	2.6	1.45	0.179
	3	5.1	2.5	1.74	0.293
	4	7.1	2.0	2.39	0.297
	5	6.6	-1.5	2.42	0.273
1931 {	2	2.2	2.2	1.19	0.193
	3	3.5	1.3	1.33	0.263
	4	5.5	2.0	1.76	0.313
	5	6.0	0.5	2.02	0.297

The time at which any significant difference appeared between the calcium absorbed by plants grown with or without boron depended, as will be seen in a later section, on the frequency with which the culture solution was renewed.

## (2) Uptake of calcium per cent. dry matter.

If the calcium taken up is reckoned as a percentage of the dry matter, a stricter comparison is possible with the results produced from plants grown with boron already described. On this basis the absorption from unrenewed solutions without boron is seen to increase until the third or fourth week only, i.e. as far as the 1930 experiment was concerned the maximum tended to be reached somewhat earlier and was significantly lower than where boron was supplied, or, in other words, a lack of boron affected the uptake of calcium more seriously than the production of dry matter (cf. Tables II and V).

As regards older plants, Table III shows that with solutions renewed fortnightly the calcium absorbed per cent. of dry matter produced was approximately the same during the sixth to ninth week, as it had been during the previous five-week period, in contrast to that which obtained with the boron-treated set. A lack of boron, therefore, had its greatest influence

during the latter part of the growth period when it depressed the uptake of calcium more than the production of dry weight. The final value after nine weeks' growth, for the plants with solutions renewed weekly, appears entirely anomalous (Table IV), as it was considerably larger than that obtained in the first five-week period for the other plants grown without boron (Tables III and V), and in consequence is apparently contradictory to the findings of Table IV, where no increase was obtained after the fifth week of growth. The explanation probably lies in the fact that the rate of renewal of the solution has an important bearing on the quantity of calcium absorbed, particularly in the case of plants grown without boron.

#### B. CALCIUM ABSORBED FROM A SOLUTION OF CALCIUM CHLORIDE ONLY.

In order to study the uptake of calcium in the simplest manner possible, and previous experience having shown that healthy growth could be maintained for a limited period under such conditions, ten plants were set up in a pure solution of calcium chloride, each receiving the same amount of calcium per plant (52.49 mg.) as the series in the complete nutrient medium had done. As before, one half of the plants were given 1 p.p.m. boric acid in addition.

##### (a) *Boron supplied.*

The quantity of calcium absorbed was considerable, the amount taken up after four weeks closely resembling that removed by the more actively growing plants in complete nutrients during a three-week period. The dry weight laid down by the latter in this time, however, was distinctly larger, so that the relative absorbing capacity of the plants in the pure solution was the greater (Table VI).

##### (b) *No boron.*

The rapidity with which the symptoms of boron deficiency appeared was striking, the inhibitory effect on root growth being particularly well marked. The earliness with which dying set in was reflected in the very small quantity of calcium absorbed, the difference between the uptake of plants grown with and without boron being much more marked in the set in the pure calcium chloride solution than in those plants grown in the complete nutrient medium. An illustration of this is given in Table VI. If, for example, the stage at which the plants with boron had each absorbed approximately 8 mg. calcium is taken as a standard, it will be seen that during the same interval the set in the pure solution, without boron, had taken up only 2.39 mg., whereas given full nutrients the corresponding uptake in the absence of boron was 5.11 mg., or rather more than twice the



same amount absorbed from the single salt solution. Indeed, comparison has to be made between plants with one week difference in age in the complete nutrients, in order to get as marked a distinction between those grown with and without boron. The fact that the absorption of 8 mg. was effected during a slightly different period under the two different nutrient conditions does not alter the question. The calcium absorbed from the pure solution per cent. of dry weight laid down was also much reduced in the absence of boron, so that a lack of this element was clearly exerting a greater inhibitory influence on calcium absorption than on dry matter production. The reduction in the root surface, however, may partly account for this, as the short, thick roots characteristic of the plants grown without boron would possess a smaller surface-absorbing area than the finely divided root system of the normal plant, and dry-weight figures would not give any indication of such differences.

TABLE VI.

*Comparison between the Uptake of Calcium from a Solution of Calcium Chloride and a Complete Nutrient Medium, the Calcium Supplied being Identical.*

	Age weeks.	Total Ca absorbed. mg.	Dry weight. gram.	Uptake Ca per cent. dry weight.	
+ B	4	8.48	1.37	0.61	Calcium chloride solution only.
No B	4	2.39	1.17	0.20	
+ B	3	8.26	2.09	0.39	Complete nutrients.
No B	3	5.11	1.74	0.29	
No B	2	2.87	1.45	0.20	

The calcium absorbed per cent. of dry weight produced was, therefore, reduced by the presence of other nutrients where boron was supplied. At the same time the appearance of the deficiency symptoms was delayed if it were withheld, so that some association between boron and calcium is suggested.

#### C. EFFECT OF THE FREQUENCY OF RENEWAL OF THE NUTRIENT SOLUTION ON THE QUANTITY OF CALCIUM ABSORBED.

##### (a) Boron supplied.

As is evident from Table VII, the total amount of calcium absorbed over a nine-week period in the presence of boron was practically identical whether the solution was renewed at weekly or fortnightly intervals.

TABLE VII.

*Effect of Frequency of Renewal of Nutrient Solution on the Absorption of Calcium over a Nine-week Period.*

Average of 5 plants.	With B.		No B.	
	Ca absorbed (mg. per plant).			
	Renewal.		Renewal.	
Period (weeks).	Weekly. <sup>1</sup>	Fortnightly. <sup>2</sup>	Weekly. <sup>1</sup>	Fortnightly. <sup>2</sup>
0-3	4.3	8.3	3.7	5.1
3-5	12.4	9.5	8.7	5.0
5-7	17.9	16.7	8.2	2.8
7-9	16.7	16.4	5.7	2.7
Total	51.3	50.9	26.3	15.6
Final dry weight per plant (gram.)	8.82	8.72	5.12	4.19
Ca absorbed per cent. dry weight	0.582	0.584	0.513	0.373
Per cent. absorption of Ca supplied	12.2	24.2	6.3	7.4

The final dry weights of the two sets of plants were also identical, so that the calcium absorbed per cent. of dry matter produced was similar in the two cases. Only in the percentage of the calcium supplied that was taken up by the plants was any significant difference in the two treatments noticeable. A more detailed investigation, however, shows that a change of solution at an early stage, such as the second week, resulted in a temporary check in calcium absorption, for the plants which remained in their original medium for the first three weeks took up approximately double the quantity of calcium in that period compared with those which were given a fresh supply at the end of the second week. It was thought possible that this check might have been partly due to the fact that such young roots were rinsed in a 2 per cent. solution of HCl before being transferred to the fresh supply of solution, but as the same effect occurred to an even more marked degree when comparison was made between older plants and those grown entirely in unrenewed solution, the change itself was probably the important factor.

The unrenewed series, on the other hand, showed a much greater calcium absorption during the first five weeks of growth than either the weekly or fortnightly series (Table VIII). Since the dry weight laid down per plant during this period was identical in the fortnightly and unrenewed sets (the figure for the weekly changed series is not available), it is evident that the plants receiving the less frequent supply of nutrients were the more active in calcium absorption.

Five weeks' growth may seem a somewhat short interval from which to deduce such results, but as the flowering stage had already been reached, a phase of rapid growth is included in this period.

<sup>1</sup> After the first two weeks.

<sup>2</sup> After the first three weeks.

TABLE VIII.

*Effect of Frequency of Renewal of Nutrient Solution on the Absorption of Calcium over a Five-week Period.*

		Solution renewed.	No. of renewals.	Total Ca absorbed per plant. mg.	Dry weight. gram.	Ca absorbed per cent. dry weight.	Percent. of supplied Ca absorbed.
+ B	{	Weekly	4	16.7	not determined	not determined	8.0
		Fortnightly	2	17.8	4.36	0.408	17.0
		Not renewed	1	25.1	4.35	0.577	47.8
No B	{	Weekly	4	12.4	not determined	not determined	5.9
		Fortnightly	2	10.1	2.80	0.361	9.6
		Not renewed	1	6.6	2.42	0.273	12.6

(b) *No boron.*

In the absence of boron entirely different results were obtained, and before attempting any explanation for this, some reference to the general behaviour of the broad bean plant grown under boron-deficiency conditions must be made. Owing to the considerable quantity of this element stored in the seed, as much as three weeks may elapse before any symptoms of a deficiency appear to the eye. The cotyledons were not removed from the plant in these experiments as the check to growth is so severe. Further, the time of the appearance of the deficiency symptoms and the rate of their progress depends on external conditions, of which the frequency of the renewal of the solutions and light (which has been the subject of a previous paper (35)) are the most important factors. The former only will be dealt with in the present instance. It has often been observed that if the nutrient solution is renewed frequently, a delay in the development of boron-deficiency symptoms generally occurs, the extreme cases being where plants are grown in 'drip' cultures (1) or in unchanged solutions. The reason for this is not quite understood unless it implies the unconscious introduction of a source of boron at each renewal of solution, but for the moment the facts will have to be accepted and allowance made for them in interpreting the results under consideration.

None of the plants grown without boron absorbed as much calcium as those supplied with it, and a further contrast in behaviour was found in that the total quantity of calcium absorbed during the nine weeks of growth was considerably lower in the series where solutions were renewed fortnightly, than in that where fresh nutrients were supplied every week, the reduction occurring chiefly during the latter part of this period (Table VII).

The amount of dry weight laid down during this time, however, was only slightly smaller in the fortnightly than in the weekly renewed series,

with the result that the uptake of calcium per cent. of dry matter was significantly greater in the plants which received a fresh supply of solution every week. Explanation for this is afforded by the behaviour of the plants grown without boron just described, for those dying the more rapidly from a lack of boron (i.e. the fortnightly renewed set) would be expected to show a greater reduction in calcium absorption than in dry matter production as the former process is the more seriously affected by a lack of boron, and further, to show any such differences from the weekly renewed series most clearly when a lack of boron had become really acute, i.e. in the later stages of growth.

A check in calcium uptake was induced by a renewal of the solution at the end of the second week of growth, similar to that described for plants supplied with boron, but as at this early stage no deficiency symptoms had yet appeared, it is not surprising that the behaviour of the two sets of plants was identical.

In the case of the unrenewed series data are available for the first five weeks only. Here definitely less calcium was absorbed than where the cultures were renewed, in contrast to the increase that obtained in the corresponding set of plants grown with boron (Table VIII). It is, therefore, evident that even during the first five weeks of growth, the presence of boron is an important factor, and that an increased rate in the development of deficiency symptoms in its absence is definitely correlated with a reduction in the frequency of the supply of nutrients, and is also reflected in an early falling off in the amount of calcium absorbed by the plant.

In a similar manner, the uptake of calcium per cent. dry weight produced was affected by the frequency of the renewal of the solution and by the presence or absence of boron, being reduced where boron was not supplied and still further reduced if the solutions were not renewed and the effect of a lack of boron thereby enhanced.

#### D. EFFECT OF THE CONCENTRATION OF CALCIUM SUPPLIED ON THE QUANTITY OF CALCIUM ABSORBED.

##### (a) *Boron supplied.*

This aspect of the problem was studied in unrenewed solutions only, and the cultures were in consequence not carried on for longer than five weeks. The quantities of calcium tested were 54.365 mg. calcium per plant (given as calcium chloride), and one-half, one-fourth, and one-eighth of this amount, a range which previous experience with the Rothamsted nutrient solution had shown included both ample and deficient supplies of this element.

Analyses were made weekly, beginning at the end of the second week, as the quantity of calcium absorbed before that time was so small as to lie

within the experimental error. From the second to the fifth week both the actual uptake of calcium (Fig. 4) and the uptake per cent. of dry matter were found to be almost directly proportional to the quantity of

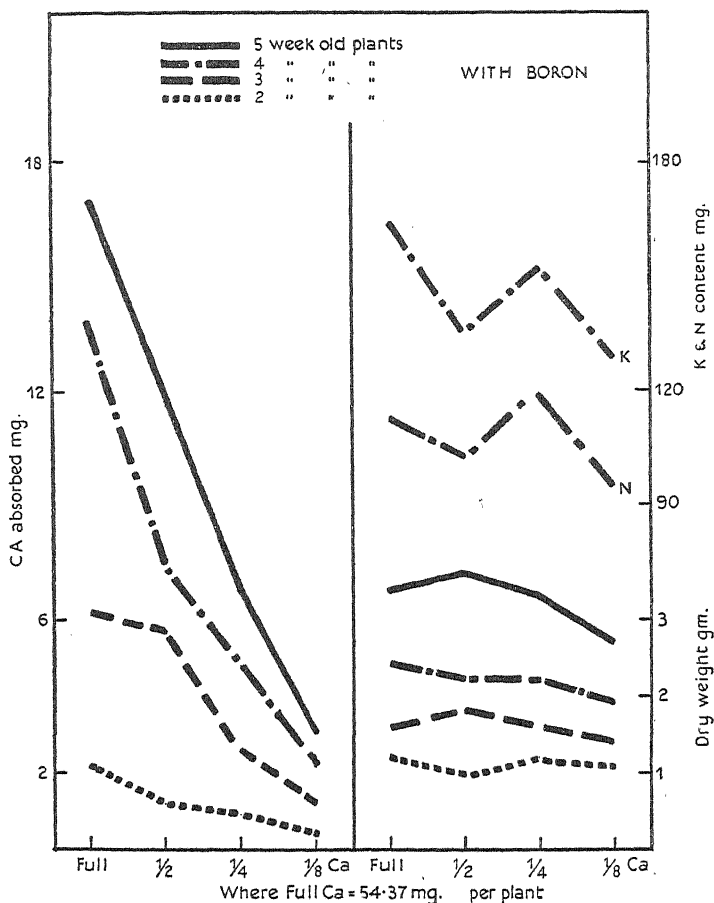


FIG. 4. Relation between calcium absorption, potassium and nitrogen content, dry matter production and the quantity of calcium supplied to *V. faba* for plants of different ages. Solutions not renewed. 1931.

calcium supplied, the course of the curves approximating to a straight line in each case. Many other workers, such as Ginsburg and Shive (11), Newton (24), and Philipson (28), have also found this direct relationship to hold with plants grown in solution cultures, while Fonder (8), Holtz (14), Shedd (34), and others report the same experience with plants in soil. Waynick's (36) and Gile and Ageton's (10) work, however, with solution and soil cultures respectively, shows that this direct relationship does not universally obtain.

The actual absorption also progressed in proportion to the age of the

plant (Fig. 5), which confirms the findings of the previous season and extends the results to smaller concentrations of calcium. By calculation it is evident that with the exception of the half dose, the period of maximum

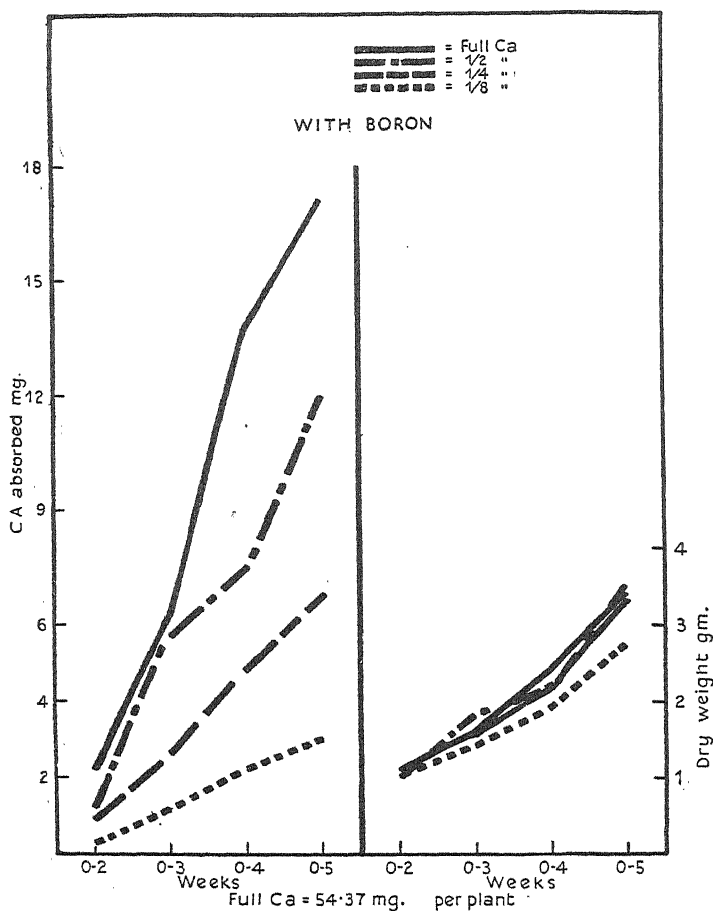


FIG. 5. Relation between calcium absorption, dry matter production, and the age of *V. faba* when supplied with different amounts of calcium. Solution not renewed. 1931.

absorption tended to lie in the fourth week, though no marked maxima occurred except where full calcium was given (Table IX). The reason for the exceptional behaviour in the plants receiving the half dose of calcium is not apparent.

Little variation was shown in the percentage of the calcium provided that was taken up by the plant in each case, except where the highest quantity of calcium was supplied. Here the percentage utilized was lower than in all other cases, which suggests that the calcium provided was in excess of that required (Table X). This view receives support from Day (6) who contends that calcium is commonly given in too high concentration

in nutrient solutions, and from Larson (17) and Hartmann and Powers (13) who found that calcium was most economically utilized when supplied at a concentration of 32 p.p.m., a value not far from that of the half dose of calcium chloride given here.

TABLE IX.

*Progress of Calcium Absorption from Nutrient Solutions Containing Different Amounts of Calcium.*

*Solution not Renewed.*

Treatment.		Calcium absorbed per plant per Week. (mg.)				
		1st. & 2nd.	3rd.	4th.	5th.	Total.
With B	Full Ca	2.2	4.0	7.6	3.3	17.1
	$\frac{1}{2}$	1.2	4.5	1.8	4.4	11.9
	$\frac{1}{4}$	0.9	1.7	2.1	2.0	6.7
	$\frac{1}{8}$	0.3	0.9	1.0	0.8	3.0
No B	Full Ca	2.2	1.3	2.0	0.5	6.0
	$\frac{1}{2}$	1.2	2.1	0.3	0.6	4.2
	$\frac{1}{4}$	0.6	1.1	0.7	-0.2 <sup>1</sup>	2.2
	$\frac{1}{8}$	0.2	0.6	0.2	0.0	1.0

TABLE X.

*Relationship between the Quantity of Calcium Supplied and Absorbed in Five Weeks. 1931.*

Total Ca supplied. mg.	With boron.		No boron.	
	Calcium absorbed.		Calcium absorbed.	
	Actual. mg.	Percentage.	Actual. mg.	Percentage.
54.37	17.1	31.5	6.0	11.0
27.18	11.9	43.8	4.2	15.5
13.59	6.7	49.3	2.2	16.2
6.80	3.0	44.1	1.0	14.7

Although the uptake of calcium bore a definite relationship to the quantity of calcium provided even from the second week of growth, the dry weight laid down did not show any such close association, but remained unaffected until the fifth week when the two smaller doses exerted a slightly depressing influence on yield (Fig. 4). It will be seen in a later section that the dry weight showed a closer correlation with the nitrogen than with the calcium content of the plant, which indicates the greater importance of the former element for dry matter production.

(b) *No boron.*

In the absence of boron the quantity of calcium absorbed throughout the five weeks' growth was approximately proportional to that supplied whether the uptake was reckoned as actual calcium (Fig. 6) or as calcium

<sup>1</sup> Negative quantity accounted for since the figure is the difference between the means values of two sets of plants which inevitably showed individual variation.

per cent. of dry matter produced. In this respect the results were similar to those where boron was supplied. The quantities absorbed, however, were very much lower than in the presence of boron except at the first

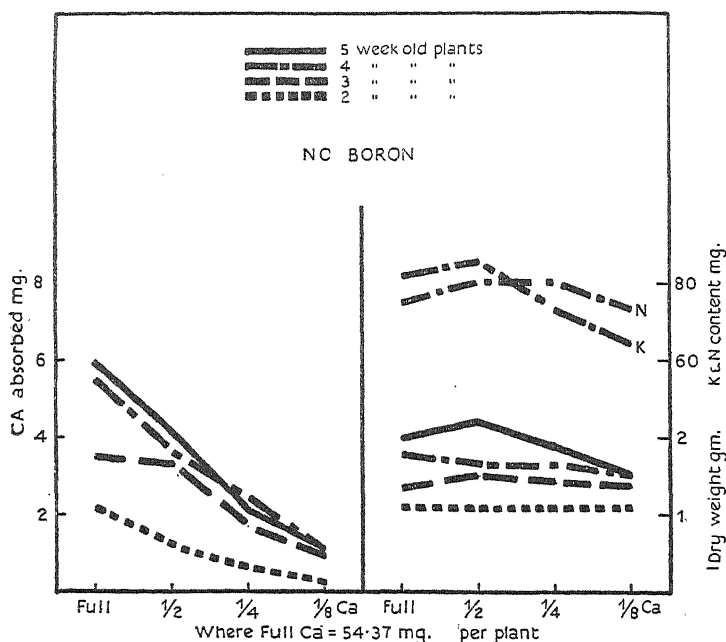


FIG. 6. Relation between calcium absorption, potassium and nitrogen content, dry matter production and the quantity of calcium supplied to *V. faba* for plants of different ages. Solutions not renewed. 1931.

analysis which was made before a deficiency of this element had arisen. The uptake slowly increased with every additional week for which the plants were grown where the two larger doses of calcium were supplied, but reached a maximum at the end of the fourth week, when only the smaller quantity of calcium was given. Except in the case of the highest concentration of calcium, the maximum absorption tended to occur during the third week, that is, a week earlier than where boron was supplied (Table IX). Although the differences between the figures are small, so that deduction from them must be made with caution, yet it is suggestive that dying from a lack of boron began to occur earlier under conditions of deficient calcium, a fact which had already been observed in previous experiments, and to which reference has been made above.

The quantity of calcium presented had little influence on the percentage that the plants absorbed, but the uptake in every case was much reduced by a lack of boron, being approximately only one-third of that which obtained where boron was also supplied (Table X).



## E. CONFIRMATORY DATA OBTAINED WITH THE USE OF SPECTROGRAPHIC METHODS OF ANALYSIS.

During the period September to December, 1928, the opportunity arose of studying spectrographic methods of analysis under Professor Lundegårdh at Experimentalfältet, Stockholm; the problem selected for investigation there was similar to that with which the present paper is concerned. Full description of the apparatus and methods have been published (20), (21), so that it suffices to state that the calcium analyses were carried out by means of flame-spectra of solutions in which the plants had been grown, after ensuring that all the calcium present was in the soluble form, i.e. as chloride. *Phaseolus multiflorus* (Blomsterböna två-fargade) was used as the experimental plant, and the basal culture solution was that employed in the present case, but the quantity of calcium supplied, though varied, was in general rather lower than in the experiments just described. Owing to the comparatively short time available and the late season of the year, no very conclusive results were obtained, although the use of artificial light and heating as the winter set in made it possible to obtain quite vigorous growth. Some brief reference to the results may, however, be of interest, for where they supply confirmatory evidence for those just described above, obtained by chemical methods of analysis, their value is considerably enhanced. In the first place both *Vicia faba* and *P. multiflorus* showed a close agreement in the very large quantity of calcium that they were able to absorb from solutions containing an adequate supply of that element. Whereas, in the presence of boron, *Vicia* plants provided with 52.49 mg. calcium per plant per change of solution absorbed on an average 40.41 mg. during fifty-three days, *Phaseolus*, similarly supplied with 36.58 mg. calcium, took up 35.08 mg. per plant in the same interval. In the absence of boron, the figures were 22.55 and 30.10 mg. per plant for *Vicia* and *Phaseolus* respectively, but as the boron deficiency symptoms developed rather more slowly in the latter species, a greater uptake was to be expected. A directly proportional relation was found between the quantity of calcium supplied and absorbed, which also confirms the results just described. Over a fifty-three-day period in the presence of boron the uptake of calcium per cent. of dry matter produced was 1.96 and 0.220 where the full quantity and one-tenth of it were supplied respectively. The corresponding figures for the plants grown without boron were 1.86 and 0.188, again showing the tendency for the uptake of calcium to be increased in the presence of boron.

## F. COMPARISON BETWEEN THE NITROGEN, POTASSIUM, AND CALCIUM CONTENT AT DIFFERENT STAGES OF GROWTH.

In order to avoid the danger of assuming too close an association between the absorption of calcium and the presence of boron in the nutrient

solution, a parallel study was made of the uptake of nitrogen and potassium by the same plants. By this means it was hoped to gain a clearer understanding as to whether the reduction in the calcium taken up by the plants grown without boron was merely the result of a general decrease in absorbing capacity, or whether it indicated a special association between boron and the amount of calcium that the plant could absorb.

(a) *Nitrogen.*

The question of a possible relationship between calcium and nitrogen in nutrition has received attention from several investigators. Gile and Ageton (10) in field experiments found no change in nitrogen content of the crop when the soil was limed, and, similarly, neither Ginsburg and Shive (11) nor Philipson (28), using water-culture methods record any close association between the calcium supplied and the nitrogen absorbed. Newton (24) studied the question from the reverse point of view, but came to similar conclusions, as peas grown with an increased nitrogen supply did not show a correspondingly higher calcium content, although their nitrogen content was raised. Parker and Truog (26), on the other hand, consider that a definite relationship between the two elements does exist and Lipman and Blair (18) found an increased nitrogen content in soy bean where lime had been applied. Further, Nightingale et al. (25) working with the tomato, showed that an absence of calcium from the nutrient medium inhibited the uptake of nitrogen, and also that the nitrogen that was absorbed remained unelaborated as the deficiency of calcium had resulted in a lack of the necessary reductase. Jacobson and Swanback (15) claim that the form in which the nitrogen is presented influences the uptake of calcium, a higher percentage of calcium being found in plants grown with nitrogen in the form of nitrate than where it is given as ammonia.

The principal question in the present experiments, however, was to determine if there were any difference in the relation between the calcium and nitrogen absorbed by plants grown with and without boron.

The nitrogen estimations<sup>1</sup> were made by the Kjeldahl method on the dried plant material, and not determined from analysis of the nutrient solution as in the case of calcium. In order to obtain comparable values of the calcium and nitrogen estimations, one of two courses was open, (a) to subtract the initial nitrogen content of the seed from that found in the plant so as to give the nitrogen in terms of uptake or, (β) to add the initial calcium content of the seed to that absorbed to give the calcium in terms of content. The latter is clearly the more accurate method, as the seed contained so much less calcium than nitrogen (1.8 or 2 mg. calcium as against 60 or 70 mg. nitrogen per seed), and all the figures quoted have been obtained in this manner.

<sup>1</sup> Carried out by the Chemical Department of the Rothamsted Experimental Station.

(1) *Boron supplied.*

A very marked decrease in N/Ca ratio occurred as the plant developed. After nine weeks' growth the ratio had fallen to approximately one-seventh of that originally found in the seed (Table XI), and the values for the successive stages of growth in plants up to the age of five weeks (Table XII), show the same course of events provided the calcium supply was adequate.

TABLE XI.

*Actual Calcium and Nitrogen Content of Vicia faba Seed Compared with that of Plants in Flower Grown in a Complete Nutrient Solution Renewed Weekly. 1930.*

	Ca.	N.	N/Ca.
	mg. per plant.		
Seed	2.0	71.8	35.9
With Boron } after 9 weeks	53.5	293.0	5.48
No Boron }	28.25	173.0	6.12

The figures for the nine- and five-week-old plants (Tables XI and XII respectively) are, however, not strictly comparable, as in the former case the solutions were renewed weekly and the supply of calcium or nitrogen was far in excess of the plants' needs, whereas in the latter instance growth was in unrenewed solutions and the plants were probably subjected to some limitation in nitrogen supply, though no actual chlorosis appeared (Table XIII). The drop in N/Ca ratio in the latter instance, therefore, would tend to be exaggerated, but the closely parallel figures obtained in the succeeding year where no shortage of nitrogen occurred (20 per cent. nitrogen still unabsorbed after five weeks) suggests that the values as given were probably but little affected by any limitation of food supplies.

Where the supply of calcium was extremely deficient, however, the fall in the N/Ca ratio with age was but small, since, although the calcium uptake was at a very much lower level throughout than where the full supply of calcium was given, the nitrogen absorption was only slightly reduced (Table XII). In fact, no correlation was found between the quantity of calcium supplied and the nitrogen content of the plant, as Figs. 4 and 8 show in the case of the four-week-old plants for actual and percentage figures respectively. The nitrogen content, however, corresponded closely with the dry weight production irrespective of the amount of calcium supplied, the correlation, with a single exception, being significant throughout the five weeks' growth. The drop in nitrogen content with the half-dose of calcium is probably partly to be accounted for by individual variation, but it will be noticed that it is also accompanied by a slight fall in dry weight and potash content.

TABLE XII. Calcium, Nitrogen, and Potassium Content of Plants of Different Ages Supplied with Complete and Deficient Calcium. With Boron.

(Solution not renewed.)

Age. Weeks.	Calcium.			Nitrogen.			Potassium.			N/Ca.	K/Ca.				
	Actual (mg.).	Percent. of dry matter.		Actual (mg.).	Percent. of dry matter.		Actual (mg.).	Percent. of dry matter.							
		Full Ca.	$\frac{1}{3}$ Ca.		Full Ca.	$\frac{1}{3}$ Ca.		Full Ca.	$\frac{1}{3}$ Ca.						
1930	Seed	2.0	—	0.125	—	71.8	—	4.46	—	—	—	—	—	Full Ca.	$\frac{1}{3}$ Ca.
	2	3.8	—	0.266	—	85.8	—	6.02	—	—	—	—	—	—	—
	3	10.3	—	0.494	—	110.6	—	5.30	—	—	—	—	—	—	—
	4	17.1	—	0.543	—	150.3	—	4.78	—	—	—	—	—	—	—
	5	27.1	—	0.623	—	146.5	—	3.37	—	—	—	—	—	—	—
1931	Seed	1.8	1.8	0.127	0.127	61.1	4.50	4.50	16.95	1.25	1.25	33.9	33.9	9.4	9.4
	2	4.0	2.1	0.342	0.191	59.8	65.0	5.21	5.82	50.74	—	4.41	—	15.0	12.7
	3	8.0	3.0	0.509	0.214	83.4	75.3	5.31	5.35	93.63	85.10	5.96	6.05	10.4	25.1
	4	15.6	4.0	0.643	0.209	113.0	94.5	4.66	4.95	164.20	127.90	6.77	6.70	7.2	23.6
	3	18.9	4.8	0.558	0.179	126.3	124.6	3.73	4.63	189.76	166.90	5.60	6.20	6.7	26.0

TABLE XIII.

*Comparison between the Calcium and Nitrogen Supplied and Absorbed under Different Conditions of Nutrition. 1930.*

	Solutions renewed.	Age weeks.	Total Ca per plant.		% Ca used.
			Supplied.	Absorbed.	
			mg.		
With Boron	Weekly	9	419.92	51.3	12.2
	Not renewed	5	52.49	25.1	47.8
No Boron	Weekly	9	419.92	26.3	6.3
	Not renewed	5	52.49	6.6	12.6

	Solutions Renewed.	Age weeks.	Total N per plant.		% N used.
			Supplied.	Absorbed. <sup>1</sup>	
			mg.		
With Boron	Weekly	9	665.6	121.2	18.2
	Not renewed	5	83.2	74.7	89.8
No Boron	Weekly	9	665.6	102.2	15.4
	Not renewed	5	83.2	20.1	24.2

(2) *No boron.*

In the absence of boron a similar fall in the N/Ca ratio occurred as the plant developed from the seed (Table XI), but the decrease was slightly less than where boron was supplied. The tendency for the ratio to be higher is also noticed in the case of the unrenewed series, where a lack of boron exerted a maximum effect (cf. Tables XII and XIV). No question of any shortage of calcium or nitrogen supply arose here, for as will be seen from Table XIII, only a small proportion of the calcium and nitrogen presented had been taken up by the plant. Under conditions where a shortage of calcium obtained, both the nitrogen and calcium contents failed to rise further after the third week of growth, but as in the case of plants supplied with boron, the nitrogen uptake was much less reduced by a shortage of calcium than was the calcium absorption, so that the N/Ca ratio was throughout considerably higher than in the plants grown in the complete nutrient solution (Table XIV). The depressing effect of an absence of boron was, further, more marked on the calcium than on the nitrogen uptake, with the result that N/Ca ratio tended to be higher in the set without than with boron, though this was not so clearly defined as where the calcium supply was adequate. The nitrogen content showed no correlation with the quantity of calcium provided (Figs. 6 and 8), and although growth was so poor in the boron-starved plants that a statistically significant association between the nitrogen content and the dry matter production could not be obtained, yet the results suggested that this held very much in the same way as for the plants supplied with boron.

<sup>1</sup> Figure obtained by subtracting original nitrogen content of seed from nitrogen found in plant.

TABLE XIV. Calcium, Nitrogen, and Potassium Content of Plants of Different Ages Supplied with Complete and Deficient Calcium. No Boron.

(Solution not renewed.)

Age Weeks.	Calcium.		Nitrogen.		Potassium.		N/Ca.	K/Ca.									
	Actual (mg.).	Percent. of dry matter.	Actual (mg.)	Percent. of dry matter.	Actual (mg.).	Percent. of dry matter.											
							Full Ca.	$\frac{1}{3}$ Ca.	Full Ca.	$\frac{1}{3}$ Ca.	Full Ca.	$\frac{1}{3}$ Ca.					
1930	Seed	—	0.125	—	71.8	—	4.45	—	—	—	—						
	2	—	0.338	—	85.1	—	5.87	—	—	—	—						
	3	—	0.413	—	91.3	—	5.31	—	—	—	—						
	4	—	0.381	—	101.8	—	4.23	—	—	—	—						
	5	—	0.355	—	91.9	—	3.80	—	—	—	—						
1931	Seed	1.8	0.127	0.127	61.1	61.1	4.50	4.50	16.95	1.25	33.9	33.9	9.4	9.4			
	2	4.0	2.0	0.352	0.175	68.6	62.5	5.78	5.48	52.05	—	4.38	—	17.2	31.3	13.0	—
	3	5.3	2.7	0.398	0.290	73.5	72.5	5.52	5.42	60.65	62.4	4.55	4.61	13.9	26.8	11.4	23.1
	4	7.3	2.8	0.415	0.192	75.0	72.8	4.27	4.97	81.78	64.2	4.65	4.38	10.3	26.0	11.2	22.9
	5	7.8	2.8	0.386	0.183	81.0	72.4	4.01	4.73	87.80	62.5	4.35	4.08	10.4	25.9	11.3	22.3

(b) *Potassium*.

Considerable discussion has arisen with regard to the relation between the potassium and calcium nutrition of the plant. Ehrenberg (7), Fonder (9),

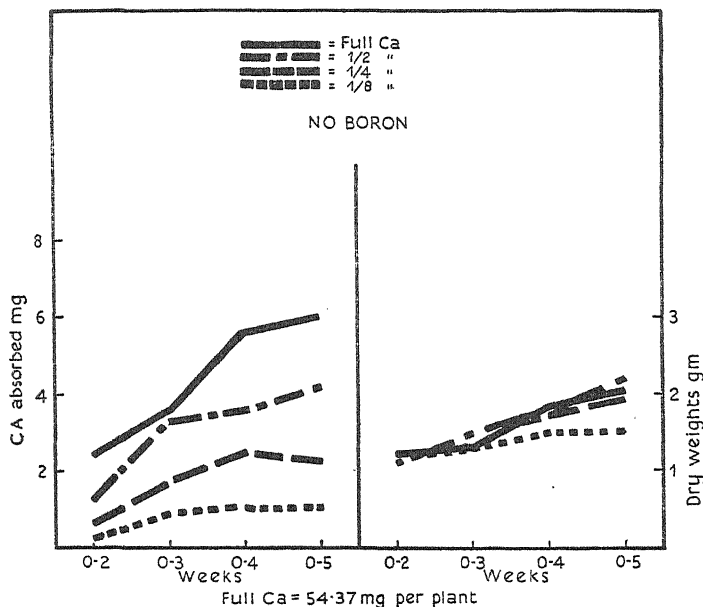


FIG. 7. Relation between calcium absorption, dry matter production, and the age of *V. faba* when supplied with different amounts of calcium. Solutions not renewed. 1931.

Lipman, Blair, and Prince (19), Sewell and Latshaw (33), and Lagatu and Maume (16) found a reduction in the quantity of potassium absorbed when large amounts of calcium were present in the soil, while Reed and Haas (32), and Newton (24), working with culture solutions, have recorded a similar inverse relationship between the two elements, the potassium content of the plant increasing if the supply of calcium was deficient. Gile and Ageton (10), and Pfeiffer and Rippel (27), on the other hand, did not find any inverse relationship to hold, and Plummer (29) even obtained an increase in potassium removed by soy beans when the soil was limed. This, however, he considered due to improved soil conditions rather than to any direct influence of the lime on the potash uptake. Colby (5) also failed to obtain any increase in potassium content in plants grown with very depleted supplies of calcium, a result he attributed to the fact that such large amounts of potassium were already present in the plant that a further rise could hardly be expected. This suggestion may well reconcile the apparently conflicting results referred to above.

In view of the interest attached to the potassium-calcium ratio, some

potassium estimations were carried out on the ash of plants in the course of the present work, four-week-old plants grown in unrenewed solutions being selected as likely to give the best-defined results. The cobaltinitrite

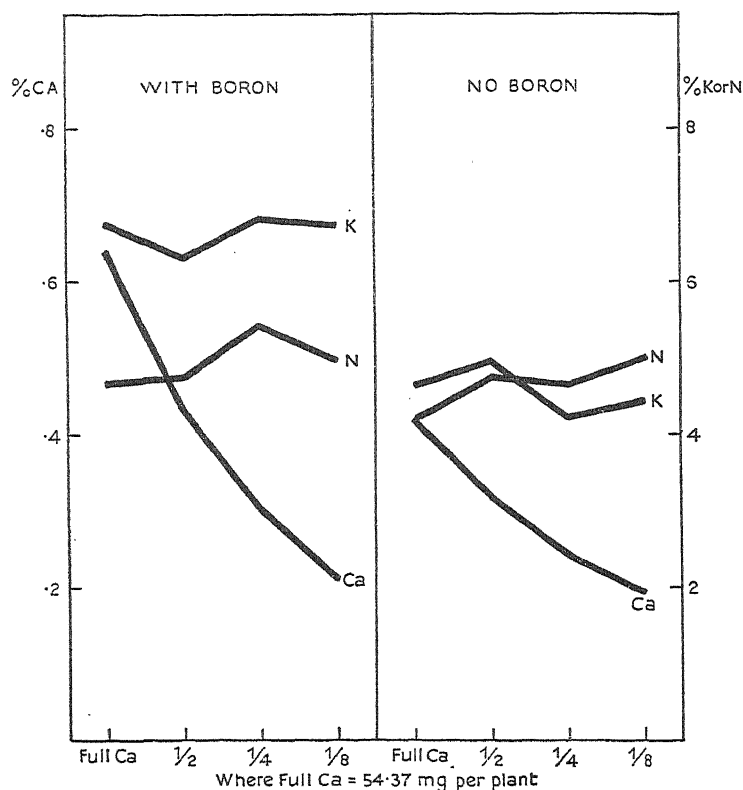


FIG. 8. Relation between the percentage calcium, potassium, and nitrogen content of *V. faba* with the quantity of calcium supplied. Age four weeks. 1931.

volumetric method was used as described by Milne (23), and the values given are the mean of closely agreeing duplicates. As in the case of the nitrogen figures, terms of content, and not absorption, have been used throughout in comparing the potash and calcium figures.

#### (1) Boron supplied.

Under full nutrient conditions the potassium, as well as the calcium content of the plants, increased with advancing age, but as the rate of increase was somewhat larger in the case of the latter element, a slight fall in the K/Ca ratio was found with time (Table XII) as Fonder (9) also described. The drop, however, was much less marked than was the case with the N/Ca ratio. No question of shortage of available potash comes into play here, as even after five weeks' growth only 50 per cent. of the potash supplied had been absorbed. In the presence of a deficient calcium



supply (one-eighth full quantity, or 6.80 mg. calcium per plant), the uptake of potassium was amazingly little reduced, in fact the dry weight tended to be more adversely affected as the slightly higher value for the percentage potassium in the five-week-old plants shows (Table XII). In consequence the K/Ca ratio showed a rise instead of a fall where the calcium supply was deficient. No definite correlation was found between the calcium supplied and the amount of potash absorbed by four-week-old plants (Figs. 4 and 8), though there is some suggestion that an inverse relationship was beginning to make its appearance after a further weeks' growth, as by then the percentage of potassium was slightly higher in the plants grown in the medium deficient in calcium than in the complete nutrient solution. Colby's explanation (to which reference has already been made) may be applicable here as the salt content of the ash of plants grown in water cultures is high, but Lundegårdh's results with wheat (21, Fig. 41) suggest that within the limits of the concentration of calcium used in the present experiments, no very close association between the calcium provided and the potash absorbed is to be expected, and that only with larger concentrations of calcium would the inverse relationship between the two elements be at all clearly defined. A further point of interest arises here in connexion with Lundegårdh's work, as he found that on an average the potassium content of wheat was approximately ten times greater than the calcium content, a figure in close agreement with the present results, in spite of the fact that the latter were obtained with such a widely differing species as *Vicia faba*. Though no statistically significant correlation was found, the potash content followed the dry matter curve very closely. As a result the curves for nitrogen and potash contents were very similar, neither showing any relationship to the quantity of calcium in the solution or in the plant (Fig. 8).

(2) *No boron.*

The results obtained were similar to those already described for nitrogen. If the calcium in the nutrient solution were plentiful, both the calcium and potash contents of the plant rose with advancing age, at least up to the fourth week, though the increase was much less marked than where boron was supplied. The K/Ca ratio, however, showed no tendency to fall after the third week, a fact which suggests that the lack of boron, while reducing the absorption of both elements, was exerting the greater influence on the uptake of calcium (Table XIV). If the calcium supply was deficient, the K/Ca ratio failed to rise with time after the third week as had occurred where boron were present, though as would be expected, the level attained was much higher than where the quantity of calcium supplied was larger (Table XIV). A lack of boron, therefore, was reducing the absorbing power of the plant for potassium as well as for calcium as

has been stated above, and further, affected the uptake of potash more than the uptake of nitrogen. The potassium content of four-week-old plants showed little, if any, correlation with the amount of calcium supplied in the solution (Fig. 6), and what trace of relationship is to be seen is of a direct rather than an inverse nature, so the results are in general agreement with those from plants grown with boron.

A deficiency of boron, therefore, tended to reduce the uptake of calcium more than that of nitrogen or potash, which would seem to indicate a closer relationship between boron and calcium, than between boron and nitrogen, or boron and potassium. At the same time it must be borne in mind that nitrogen and potassium are generally most vigorously absorbed during the early stages of growth, whereas calcium is still freely taken up at later stages of development just when the effects of any deficiency of boron have reached their full importance. Further, a lack of boron reduces the development of the root more seriously than that of the shoot, so that a diminished capacity for absorption of all nutrients inevitably results when boron is withheld.

### III. DISCUSSION.

A consideration of the foregoing results shows that the quantity of calcium a plant absorbs is influenced by a number of factors. The age of the plant is a matter of importance, for in general the absorption increases with age up to quite a late phase of development, though some periodicity in uptake is also evinced. In this connexion the necessity for carrying on experiments for sufficiently long periods, if a true picture of the events is to be secured, is clearly brought out. Another factor upon which the extent of the absorption of calcium depends is the quantity of the calcium supplied, and although certain concentrations appear to be more economically utilized than others, the uptake is approximately proportional to the amount supplied. Based upon dry matter production, the absorption of calcium is greater from a pure solution of calcium chloride than from complete nutrients, and a similarly reduced uptake is obtained when the nutrient medium is frequently renewed, that is to say, the absorption of calcium is affected by the presence and quantity of other elements.

The question as to whether or not any association exists between boron and calcium uptake must, therefore, be considered under a variety of conditions. The approach to the problem, however, is rendered particularly difficult by the fact that death ensues in the absence of boron, and in consequence all metabolic processes receive a check, and any condition which hastens or retards the onset of the boron deficiency symptoms will inevitably alter the absorbing capacity of the plant. Some points, however, stand out distinctly. In the first place certain main results hold good irrespective of whether boron is present or not, the differences being those

of degree only. For example, a proportional relationship between the calcium supplied and absorbed, and an increased uptake as the plant developed is found in both cases, though the quantities absorbed in the absence of boron are considerably the smaller. In the second place, other results are dependent upon whether the plants are grown with or without boron, but such differences can almost always be traced to the effect of the nutritive conditions on the rapidity with which boron deficiency symptoms appear. An example of this is found in the greater absorption of calcium from unrenewed than from frequently renewed solutions when boron is present, whereas in the absence of boron the reverse is the case. This apparently direct association between the reduction in calcium uptake and the lack of boron has, therefore, to be viewed in the light of the plants' capacity to absorb other elements, i.e. allowance must be made for the general reduced vitality of the plant in the absence of boron, before any conclusions can be drawn. Both the potassium and the nitrogen contents are much less affected by the absence of boron than is the calcium content, so that an affinity between boron and calcium is certainly suggested. The data which point to such an association most strongly, however, are those obtained firstly, from comparisons of plants grown in complete nutrients with those in a pure solution of calcium chloride, and secondly, between those grown in unrenewed and frequently renewed solutions. In both cases the conditions which delayed the onset of the boron deficiency symptoms (i.e. the presence of complete nutrients and their frequent renewal respectively) also inhibited the uptake of calcium in the presence of boron, facts which certainly point to an affinity between the two elements, for if boron were needed for the uptake of calcium, and its supply limited to that originally provided by the seed, any condition which would tend to increase the absorption of calcium, such as the absence of other nutrients, would also hasten the appearance of boron deficiency symptoms. It is, therefore, of particular interest that somewhat similar views should have been expressed in two recent publications, though the question was approached from rather a different aspect than has been the case in the present paper. Haas (12), in a study of the effect of toxic quantities of boron on fruit trees, showed that the presence of excess of this element resulted in a decreased absorption of calcium by citrus and walnut. Bobko and Belvoussev (2), on the other hand, found that injury to sugar beet induced by excessive calcium in the soil could be corrected, and in fact the calcium turned to good account, by the application of small quantities of boron. Excess of either element, therefore, affected the need of the plant for the other. At the same time, it has to be borne in mind that although the behaviour of elements needed in such small quantities as boron may well differ from those required in larger amounts, yet in general no one element bears a unique relationship to one other element, excess or

deficiency of any individual nutrient usually bringing about a number of changes, both chemical and physiological, owing to the displacement of the balance between all the components of the plant's food.

The evidence so far obtained, though insufficient to provide any definite proof of an association between boron and calcium, does, however, suggest that a relationship of some sort exists between them, the exact nature of which is as yet undetermined.

#### IV. SUMMARY.

1. Over a nine-week period the quantity of calcium absorbed per week by *Vicia faba* increased up to the seventh or fourth week respectively, according as to whether or not a trace of boron was present, indications of periodicity in uptake being obtained in the former case.

2. Less calcium was absorbed from solutions renewed at weekly or fortnightly intervals, than from unrenewed solutions when boron was present, but the reverse was the case if boron were not provided. This difference is attributed to the fact that renewal of the solution delays the appearance of the boron deficiency symptoms, and thus prolongs the absorbing capacity of the plant.

3. The quantity of calcium absorbed was approximately proportional to the calcium supplied, irrespective of the presence or absence of boron, although the total calcium taken up was much reduced under the latter condition.

4. No correlation was found between the calcium supplied and the nitrogen or potash content of the plant, both the latter showing a closer affinity with the production of dry matter.

5. Under full nutrient conditions the N/Ca and K/Ca ratios in the plant fell as its age increased, the fall being more marked in the presence than in the absence of boron. A lack of boron, therefore, reduced the uptake of calcium more than that of nitrogen or potash.

6. In the presence of boron, the calcium absorbed per unit dry matter produced was higher from a pure solution of calcium chloride, than from a complete nutrient medium containing a similar quantity of calcium. In the absence of boron, death ensued the more rapidly in the plants grown in the single salt solution, so that the presence of other nutrients apparently increased the requirement of the plant for both calcium and boron. Although the evidence is not conclusive, indications of an association between boron and calcium were, therefore, obtained.

In conclusion, thanks are due to Mr. R. G. Warren for his helpful advice with regard to the chemical methods employed, and to Professor J. G. Thorsgårdh for his generosity in placing his valuable experience and laboratory facilities at the writer's disposal.

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# The Composition of the Salts on the Leaves of Some Desert Plants.

BY

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VOLKENS (2) considered that the salts found on the leaves of *Reaumuria hirtella* and other desert plants were part of a mechanism for the absorption of water or water vapour from a saturated or nearly saturated atmosphere by the aerial parts of these plants. He performed no analyses, but noted that the crystals were largely cubical, indicating probably sodium chloride.

Marloth (1) investigated the salts on *Tamarix* (species not given). Table III has been recalculated from his results, to show ions instead of molecules.

Williams (3) seems to have assumed from the cubical shape of some of the crystals that the salt was pure sodium chloride; he does not give an analysis. It is difficult to see how plant cells could excrete pure sodium chloride from the mixture of salts in the cells.

## MATERIAL.

The material investigated was *R. hirtella*, from Wadi Digla, twelve miles south of Cairo, and *Tamarix arborea* and *Cressa cretica* from Gebel Asfar, seventeen miles north-east of Cairo. The Wadi is a very dry valley, with rock a few centimetres below the surface of the sand. Gebel Asfar is a flat area in the desert, watered by Cairo drainage water, the level of the water in the drains being about one metre below the general surface level. This area was pure desert before the drainage water was brought there, and *Tamarix* and *Cressa* are survivors from the desert flora. The rainfall of both areas is under twenty-five mm. per annum.

## EXPERIMENTAL METHODS.

It was found very difficult to wet the leaves of *Reaumuria*, which is a low, bushy shrub, something like *Calluna vulgaris*, with very small leaves. Finally, the shoots were put into water at 40° C. for ten to fifteen minutes. The contents of the living cells were not extracted, as shown by the total absence of carbohydrates in the samples obtained. The solutions thus obtained were evaporated to dryness at 80° C. Two samples of

*Reaumuria* were obtained from plants collected within a distance of two miles along the valley, and except for sulphate and chloride ions, the two were approximately similar. On the other hand, the salts from *Tamarix* and *Cressa* differed more from each other, though collected within an area of a few square yards. (Thirteen litres of salt extract from the leaves gave about 20 grm. of salts.)

TABLE I.

*Analyses of the Salts on the Leaves of Reaumuria hirtella.*

	First sample.	Second sample.
	%.	%.
Calcium (Ca)	7.1	4.13
Magnesium (Mg)	1.7	1.20
Potassium (K)	0.9	0.68
Sodium (Na)	20.0	26.79
Iron (Fe)	0.1	0.03
Chloride (Cl)	27.7	44.54
Sulphate (SO <sub>4</sub> )	19.4	5.85
Silicon (Si)	0.2	0.07
Loss at 120° C.	4.9	7.27
Loss on ignition	17.1	—
Phosphate (PO <sub>4</sub> )	a trace	nil
Nitrate (NO <sub>3</sub> )	not appreciable	nil
Nitrite (NO <sub>2</sub> )	—	nil
Ammonium (NH <sub>4</sub> )	—	nil
Borate (BO <sub>3</sub> )	—	nil
Bromide (Br)	—	nil
Iodide (I)	—	nil
Sugar	nil	nil
Starch	nil	nil
Manganese (Mn)	—	< 0.01
Aluminium (Al)	—	< 0.01
Carbonate (CO <sub>3</sub> )	nil	nil
Acetate	nil	—
Oxalate	nil	—
Succinate	nil	—

## DISCUSSION OF RESULTS.

The salts on *Reaumuria* (first sample) consisted chiefly of sodium, calcium, chlorine, and sulphate ions. Magnesium, potassium, and iron were present in small quantities, phosphate was present only as a trace, whilst nitrate, carbonate, acetate, oxalate, succinate, and carbohydrates were entirely absent. (The soil here is derived from magnesium limestone, and contains much calcium and magnesium.) The second sample contained less calcium and sulphate ions, and more sodium and much more chloride. Magnesium and potassium were again present in small quantities, while the following were absent or present in minute quantities: iron, silicon, phosphate, nitrate, nitrite, ammonium, borate, bromide, iodide, carbohydrates, manganese, aluminium, and carbonate.

The organic acid (or acids) present was not identified, but it was found to be unsaturated, sintered at 160° C., first melted at 170°, then wholly



melted at  $188^{\circ}$ ; it was probably a mixture of organic acids. The organic matter of sample 2 contained 0.12 per cent. of nitrogen, calculated as a percentage of the original weight. There was also an excess of sulphur of 0.07 per cent. over and above the sulphate present.

TABLE II.

*Analyses of the Salts on the Leaves of Tamarix arborea and Cressa cretica.*

	Tamarix.	Cressa.
	%.	%.
Calcium (Ca)	8.11	1.26
Magnesium (Mn)	1.08	1.32
Potassium (K)	1.96	0.83
Sodium (Na)	16.63	23.77
Iron (Fe)	0.13	0.19
Chloride (Cl)	16.19	25.00
Sulphate (SO <sub>4</sub> )	31.26	13.26
Silicon (Si)	0.11	0.12
Loss at 120/130° C.	8.33	13.40
Carbonate (CO <sub>3</sub> )	small	small
Nitrate (NO <sub>3</sub> )	small	small
Nitrite (NO <sub>2</sub> )	traces	traces
Phosphate (PO <sub>4</sub> )	slight traces	slight traces
Ammonium (NH <sub>4</sub> )	slight traces	slight traces
Borate (BO <sub>4</sub> )	slight traces	slight traces
Organic acids	not determined	not determined

Table II shows that the salts on the leaves of *T. arborea* contained ions similar to those of the *Reaumuria* salts, but in rather different proportions. Sodium and chloride were lower, but sulphate was much higher; magnesium and iron were about the same, but potassium was rather higher; carbonate and nitrate were present in small but appreciable quantities, together with traces of nitrite, phosphate, ammonium salts, and borates. Organic acid radicles were again present (shown by smell and alkalinity on charring), possibly as valeric acid.

The salts on *Cressa cretica*, though the plants were living within a few yards of *Tamarix*, showed considerable differences from the salts on the leaves of that plant. There was much less calcium and sulphate, and considerably more sodium and chloride. Magnesium and iron were about the same, but potassium was less. Carbonate and nitrate were again present in small but appreciable quantities, together with traces of nitrite, phosphate, ammonium, and borate. Manganese was absent from both samples. Unknown organic acids were again present.

Marloth (1) found on the leaves of *Tamarix* (species not given) a mixture of salts consisting chiefly of calcium and carbonate ions. Magnesium was higher than on *Tamarix arborea*, but sodium, sulphate, and chloride were much lower. Potassium and iron were absent, but much nitrate and considerable quantities of phosphate were found.

TABLE III.

*Analysis of the Salts on the Leaves of Tamarix (Data after Marloth but Recalculated as Ions.)*

	%.
Calcium (Ca)	20.8
Magnesium (Mg)	3.0
Potassium (K)	nil
Sodium (Na)	8.5
Iron (Fe)	nil
Chloride (Cl)	6.8
Sulphate (SO <sub>4</sub> )	4.7
Silicon (Si)	nil
Water	6.1
Phosphate (PO <sub>4</sub> )	2.6
Nitrate (NO <sub>3</sub> )	12.6
Carbonate (CO <sub>3</sub> )	33.3

## CONCLUSIONS.

On the whole, the salts on the leaves under consideration consist of those of least use to the plant (except calcium and sulphate). Thus we have much sodium and chlorine, and little or no potassium, magnesium, iron, nitrogen, and phosphorus. Carbohydrates were entirely absent, but there were small quantities of an undetermined organic acid. The leaves therefore would appear to excrete ions of little value to the plant, and to hold back those which are essential. Marloth's analysis of the salts of *Tamarix* showed the presence of large quantities of insoluble calcium carbonate. It is difficult to understand how this salt could be excreted by the leaf cells.

The author is indebted to Professor F. W. Oliver, Dr. F. L. Warren, and Dr. B. Dyer for advice and assistance.

## SUMMARY.

1. Analyses are given of the salts found on the leaves of certain plants of the Egyptian desert.
2. The analyses show the presence of ions which appear to be of little use to the plant; most of the elements essential to the plant are present in very small quantities or absent.
3. No carbohydrates were found in any of the samples obtained.

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# A Contribution to the Physiology and Ecology of *Peltigera canina* and *P. polydactyla*.

BY

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With twelve Figures in the Text.

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## INTRODUCTION.

THE lichens are the most drought-resisting of all plants, with a unique power of recovery after desiccation, and their water relations have been the subject of a number of investigations. As dual organisms, dependent on the photosynthetic activity of one partner, they present special problems in carbon-assimilation, an aspect of their physiology which has also received attention. In a plant subject to serious diminution of water content, this factor becomes one of the most important in determining the rate of respiration and assimilation. The lichens are peculiarly suitable for investigations of the relations of these processes to water content; for in no other plants, except perhaps in some subaerial algae, does water content vary so widely with no more serious disturbance to normal metabolism than a temporary change in the ratio of the two.

Zukal (34) and Sievers (28) investigated the water relations of lichens. Both hold that the upper surface is 'protective'. In some species there is a secretion of lichen acids, which prevents wilting of the surface, and so hinders absorption and also evaporation. In others the protection is ascribed to the thickness of the close upper cortex. By timing the rates of absorption of drops of water by the upper and lower surface, Sievers

found that the latter was more efficient. Zukal found that lichens could absorb considerable amounts of water from water vapour. Göebel (15) came to the conclusion that the walls of the hyphae were important in retaining water by colloidal imbibition. He attributed to the presence of lichen acids the difficulty of wetting the intervenal regions of the lower surface of certain lichens. He drew attention to the importance for gaseous exchange of the air-filled intercellular spaces of the algal layer. The absorption of water by the lichen he considered as a purely imbibitional process, since dried lichens swell readily in water.

Bachmann (1, 2) carried out an extensive investigation of the water relations of a number of *dead* crustaceous and foliaceous lichens, growing on calcareous or siliceous rocks. He found that crustaceous lichens absorbed large amounts of water from dew and rain. The water absorbed by night from dew was lost by day after two hours in the sun or four hours in the shade. If it had rained by night, the water absorbed lasted about twice as long. In spring and autumn complete drying out did not occur. Crustaceous lichens absorbed more water weight for weight than foliose; they lost water more readily to begin with, but less readily as they approached desiccation. Endolithic lichens are most retentive of water. The presence of a layer of dead hyphae and gonidia, the 'hyponekralschicht', is recognized as important in absorbing and retaining a store of water.

Kolumbe (21) showed that lichens could absorb considerable quantities of water in the form of vapour, though, even in a saturated atmosphere, they never reached saturation. This capacity accounts for their survival, and the possibility of carbon-assimilation in dry regions of both hot and cold zones. Hiltzer (18) supported the view that water absorption in lichens is purely physical, and compared the maximum water content in a variety of species. Stocker (29) published a careful quantitative study of water relations. He states that water is held mainly outside the cells in capillary interstices and in the walls. Liquid water is absorbed with great rapidity, the lichen becoming saturated within two minutes; this is due to the rapidity of movement into the capillary spaces. The course of evaporation and of uptake of water vapour is that of typical imbibition by, and evaporation from, a gel. Drying out takes place very rapidly. The water content of a lichen in air is related to the humidity of the air. The area of a lichen is roughly proportional to its water content.

The earliest work on respiration and assimilation in lichens is that of Jumelle (20). He found that fruticose and foliose lichens showed an excess of assimilation over respiration even in diffuse light, while crustaceous lichens showed an excess in bright light only. The assimilation by the algae of the gonidial layer was therefore sufficiently active to provide a balance of material for the whole organism. Assimilation increased with water content almost to the saturation point. Dry lichens were found to

be very resistant to high temperatures; their power of assimilation returns even after exposure for 55 hours to 60° C. Assimilation was appreciable at temperatures of -32° C. though respiration ceased at -10° C.

Henrici (17) found that assimilation ratio varied with temperature and light intensity in a complicated fashion. Two or more maxima were found on increasing either of these factors. The number and position of the maxima changed with the previous treatment and with the habitat of the lichen. Unfortunately the small number of determinations makes it impossible to place much weight on these results. Fraymouth (14) found that in *Parmelia* respiration increased with water content. Boysen Jensen (7) found that in air of normal CO<sub>2</sub> content respiration balanced assimilation at the high light intensity of 4,200 lux. For the leaves of shade plants this 'compensation point' lies at 200-400 lux, and for sun leaves at about 700 lux. The high compensation is related to the high respiration of the fungal component, and the conclusion is drawn that only a very small carbon-compound balance is available for growth. Probably part of the carbon compound supply is drawn by the fungus from the substratum.

Stocker (29) found that assimilation and respiration increase at first with rising water content, but fall before saturation is reached. In the dry lichen respiration is most active. The temperature assimilation graph shows two maxima at 12° C. and 25° C. The temperature respiration graph shows maxima at 5° C. and 25° C. No complete explanation of these curious results is suggested. The shade lichen *Lobaria* has a compensation point at 150 lux, the sun lichen *Umbilicaria* at 2,500 lux.

The object of the present work was to make a detailed study of the water relations, the assimilation and the respiration of a single lichen. *Peltigera canina* was chosen as a species which can be readily obtained in quantity and freed from extraneous matter without damage. The closely related *P. polydactyla* was also used and found to behave in the same way.

The structure of *P. canina* has recently been described by Darbishire (11). The lichen grows in grass or on moss in rather shady places. On drying, the flat, dark-green lobes change to a greyish colour and the lichen shrinks and curls up. About 5 per cent. of the thickness of the thallus is occupied by a close upper cortex, without intercellular spaces except those due to the little cracks which result from repeated drying. The algal layer is about 10 per cent. of the thickness of the thallus and is rich in air spaces. The remaining 85 per cent. in the region of the veins consists of close-packed parallel hyphae from which spring rhizoids which penetrate the mosses of the substratum. Between these veins the lichen is thinner and the hyphal threads are woven with abundant air-spaces which make wetting of the intervenal areas in the lower surface difficult. There is no lower cortex in this genus.

## I. WATER RELATIONS.

Stocker's results were published after much of the work on this part of the problem had been carried out. As the work confirms his, only a brief description need be given.

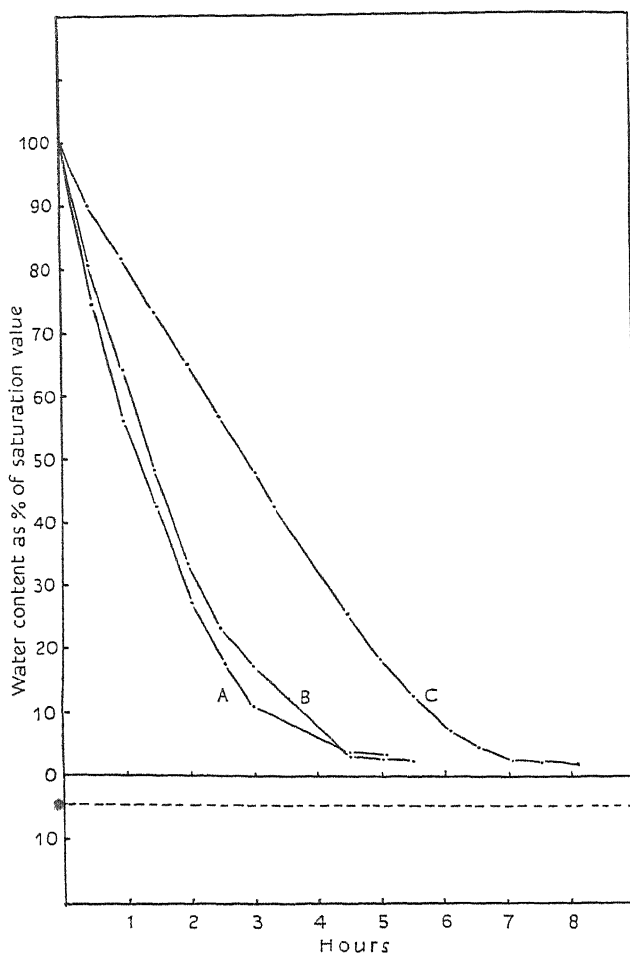


FIG. 1. Drying out of lichens and agar gel in air. A, *Peltigera canina*; B, *P. polydactyla*; C, Agar gel. Room temperature approx.  $18^{\circ}\text{C}$ . Humidity = 11.6 mm. mercury. The zero dry weight taken after 24 hours in desiccator. The broken line represents the weight after drying at  $110^{\circ}\text{C}$ .

(i) *Evaporation and absorption of water vapour.*

Evaporation was measured by weighing on a sensitive spring balance pieces of lichen suspended over calcium chloride in a thermostat at  $21^{\circ}\text{C}$ . Graphs for *P. canina*, *P. polydactyla* and a thin sheet of agar are given in Fig. 1. They are exponential in form, and the only difference is in the

much more rapid drying out of the lichen. This is in agreement with Stocker's work. The extensive intercellular spaces of *Peltigera* mean that there is only a layer of cells some 20-40  $\mu$  thick which is not in open connexion with the atmosphere. In an agar gel, on the other hand, as the surface dries out diffusion of liquid must be considerably slowed down.

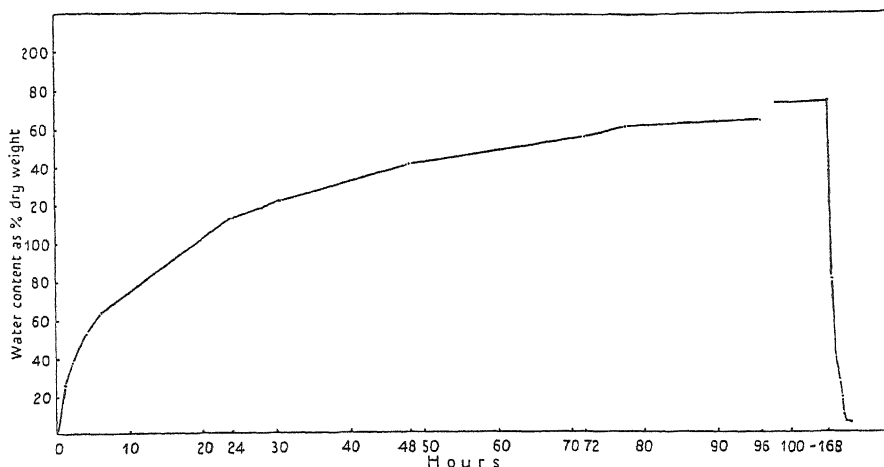


FIG. 2. Absorption of water vapour by *P. canina* during 7 days at 21° C. The graph at the right hand of the figure shows the course of drying out on the same time-scale.

Absorption of water vapour was followed by periodical weighing of portions of thallus suspended in a saturated atmosphere in jars at 21° C. The lichen could be removed from the jars in the incubator, weighed on the spring balance, and returned to the jars in less than one minute; thus no appreciable drying out occurred during manipulation. The course of absorption is shown in Fig. 2. It is a relatively slow process, a week being required to reach equilibrium as compared with 2.5 hours for complete drying out. The first half of the water is absorbed in about sixteen hours, and the relation of absorption to time is again the exponential one characteristic of the absorption of a gel.

Some figures may be given here of the actual water content of the lichen under various conditions. They are averages of a number of determinations. Considerable variation occurs, but they are useful as showing the order of water content. The dry weights were determined at 110° C.

Water content after drying at 21° over calcium chloride: 9 per cent. of dry weight or 3.4 per cent. of saturation water content.

Water content after severe drought in nature: 13 per cent. of dry weight, or 5 per cent. of saturation water content.

Water content after complete absorption in saturated atmosphere: 190 per cent. of dry weight or 71 per cent. of saturation water content.

From a moist atmosphere the lichen can thus absorb a very large amount of moisture, but only 70 per cent. of that absorbed from liquid water. It is reasonable to suppose that this 70 per cent. is water held in the walls and lumina of the hyphae, while the 30 per cent. is water held in the intercellular spaces. It is of interest to note that desiccation in nature approaches that over calcium chloride.

(ii) *Absorption by, and evaporation from, the two surfaces.*

Evaporation from the two surfaces was compared by weighing three pieces of lichen, the first with both surfaces free, the second with the upper surface vaselined, and the third with the lower surface vaselined. Some determinations were also made with two pieces of lichen, the course of evaporation being first followed with both surfaces free and then, after soaking in water, from the upper surface of one and lower surface of the other. The two methods gave concordant results and a set of graphs obtained by the first is given in Fig. 3.

Evaporation is naturally most rapid with both surfaces free and the upper surface loses water more slowly than the lower. If the water loss is plotted against the logarithms of the time, comparative values of rate of evaporation may be obtained from the slopes of the resulting straight lines. It is found that the ratio of evaporation rates from both surfaces, from lower and from upper, is as 10:8:7. The relative times for loss of 50 per cent. of the water content are 10:8.8:7.4. There seem to be no special protective features in the upper cortex of *Peltigera*; lichen acids are not produced in this genus, and the upper surface can be easily moistened by liquid water which it absorbs readily. The slower rate of evaporation from this surface is due to two factors: (i) the smooth surface offers a smaller evaporation area than the corrugated lower surface, and (ii) there is no free gaseous diffusion from the interior by intercellular spaces. In dry weather in nature evaporation through the upper surface would take more than twice as long to bring the lichen to the air-dry condition than if both surfaces were exposed, and this must be important, even though the effect is somewhat diminished by the curling up and partial exposure of the lower surface, as drying proceeds.

Similar experiments were carried out on the absorption of water vapour. The rate of absorption was less through the upper than through the lower surface at first, though after five days the amounts absorbed were equal. After forty-eight hours the ratio of absorption through both surfaces, lower and upper, were as 10:9.7:8.4. The high efficiency of the lower surface must be referred to the extensive gaseous diffusion which it allows to the thallus from below. In nature of course absorption will always take place through both surfaces.



(iii) *Living and dead material.*

Comparison was made between absorption by, and evaporation from, living lichen material and material killed at 110° C. It was found that the

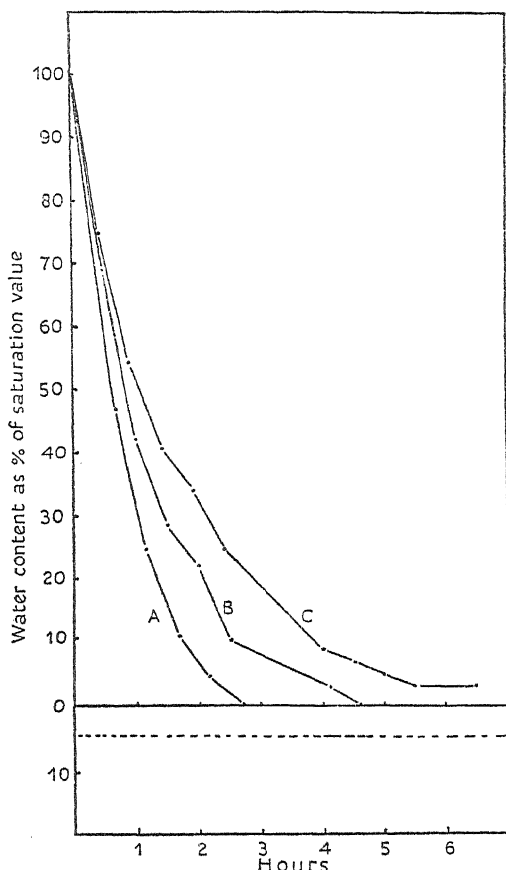


FIG. 3. Drying out of *P. canina* over  $\text{CaCl}_2$  at 21° C. A, both surfaces free; B, lower surface free, C, upper surface free. Zero water content taken after 24 hrs. The broken line represents the position of zero after drying at 110° C.

dead lichen after saturation with liquid water retained 71 per cent. of that held by living material. For *P. aphthosa* Goebel (15 a) obtained a figure of 77 per cent. Living material also absorbs rather more water as vapour. After thirty hours in a saturated atmosphere it was found that the absorption by dead material was 90 per cent. of that by the living lichen. There is no appreciable difference in the rate of evaporation from the living and the dead lichen.

The difference in absorptive power by dead and living material may be due to (a) absence of osmotic absorption, (b) alteration in the cell-wall

colloids as a result of the action of high temperature. A difference in imbibitional absorption is indicated by the difference in the power of absorbing water vapour. When the hyphae swell they also separate somewhat, leaving interstitial spaces which are filled by water, and since hyphal swelling is lessened in the dead material, the reduction in size of these spaces will help to explain the large saturation deficit of the dead material.

## II. RESPIRATION.

Measurements of respiration were made with two aims in view:

- (a) To determine the relation between respiration, temperature, and water content.
- (b) To provide a basis for estimation of real assimilation.

It proved that even carefully selected and apparently uniform material was very variable, and numerous estimations on different samples were made. The data obtained are, however, sufficient to establish the general relations.

*Method.* In estimating respiration about 3 grm. of lichen were placed in a flask immersed in a darkened water bath, the temperature of which was controlled by a toluol thermo-regulator. A current of air freed from  $\text{CO}_2$  by passing over soda-lime, and brought to the temperature of the bath in passing through an immersed glass 'worm', was drawn over the lichen by a constant-level siphon. The current was kept at 1 litre per hour. The  $\text{CO}_2$  produced was absorbed by N/50 baryta in a Pettenkofer tube and estimated by titration against N/50 HCl. One drop from the burette was equivalent to 0.044 mg.  $\text{CO}_2$ . As a rule each absorption lasted an hour with a preliminary period of half an hour before the first of a series, with intervals of ten minutes between successive absorptions.

Preliminary experiments showed that respiration rate always falls off from that of the first estimation. When the lichen which had been used for four or five determinations was returned in the evening to the cool greenhouse, where the material was kept, and then used for a further series on the following day, the initial value in the second day was lower than the final value on the first. If, however, the lichen was left in the greenhouse for several days the initial value was regained. An example of this behaviour is given in Table I. The same specimen was used throughout, returned to the greenhouse every night, and from November 15 to 20.

The falling-off cannot be a time effect due to temperature, as it occurs at the lowest temperature employed. Nor is it due to drying out, for even though a certain loss of water occurs during a series of estimations, this is not sufficient to account for the decrease in respiration obtained. It seems certain that the exhaustion of food substances must be responsible, and this is confirmed by the recovery after four days in the greenhouse, though

no recovery took place in the greenhouse over night, i.e. during a period of darkness.

TABLE I.

*Respiration of P. canina (mg. CO<sub>2</sub> per grm. fresh wt. per hour).*

Temperature 21° C.

Date.	1st Estimate.	2nd.	3rd.	4th.	5th.
Nov. 12	0.311	0.260	0.285	0.282	—
13	0.159	0.146	0.109	0.164	0.139
14	0.146	0.158 (?)	—	—	—
15	0.139	0.088	0.063	0.088	—
20	0.277	0.139	—	—	—

In the experiments on the relation of respiration to temperature, fresh material was used every day and a series of determinations made on each

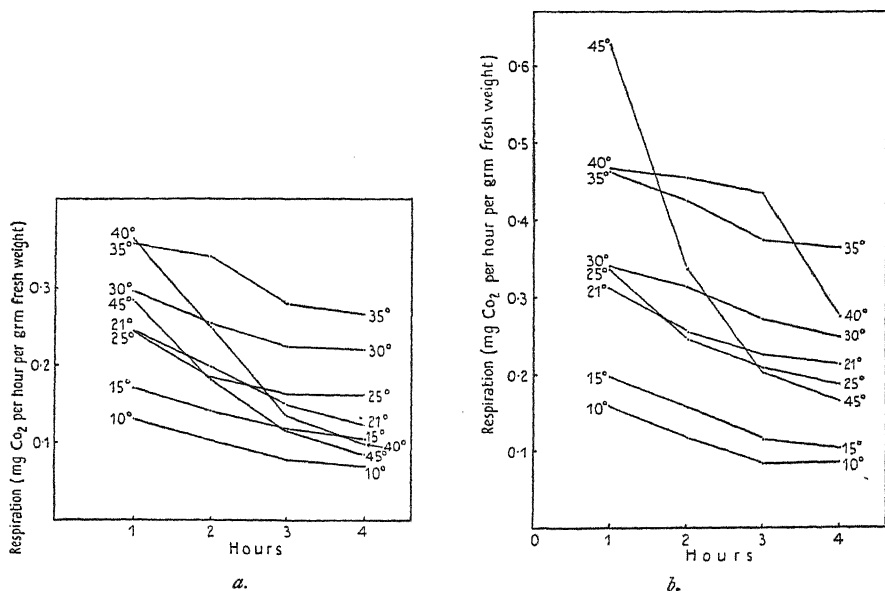


FIG. 4. (a) Respiration of *P. polydactyla* at various temperatures. (b) Respiration of *P. canina* at various temperatures.

sample. The first and fourth values in each series are recorded in Table II for each of the two species employed. The water loss, as percentage of original fresh weight at the end of each series, is also given.

Figs. 4 a and 4 b show the course of respiration with time for all the temperatures used. Several series were made for each temperature. Each point is the mean of all values obtained for any one temperature, at the same stage in the series of estimations (i.e. first, second, third, or fourth).

When working with *P. canina* at high temperatures abnormally high initial values were obtained several times. It was found that after a day this species frequently developed a growth of mould in the flasks, which, however, was not visible the first day: it is probable that the high results on the first day were due to the mould. This trouble did not occur with *P. polydactyla*, and the conclusions drawn are based primarily on the results obtained with this species.

TABLE II.

*Effect of Temperature on the Respiration of Peltigera.*

The 1st and 4th values of each set of determinations are given as mg. CO<sub>2</sub>, per grm. fresh weight, per hour. The water-loss at the end of each set is given as percentage of original fresh weight. The figures in column A represent the first estimation, and those in column B the fourth.

Temp.	<i>Peltigera canina</i> .			<i>Peltigera polydactyla</i> .		
	Respiration.		Water loss.	Respiration.		Water loss.
	A.	B.		A.	B.	
10° C.	0.189	0.104	3.37	0.180	0.096	2.80
	0.143	0.060	—	0.102	0.049	—
	0.170	0.087	1.77	0.108	0.053	1.36
	0.129	0.096	2.69	0.130	0.078	1.56
15° C.	0.210	0.107	2.50	0.186	0.137	—
	—	—	—	0.161	0.119	1.76
	0.185	0.100	2.53	0.178	0.086	2.11
	—	—	—	0.155	0.101	1.60
21° C.	0.347	0.206	—	0.300	0.217	—
	0.294	0.177	—	0.248	0.111	—
	0.366	0.246	3.30	0.279	0.129	7.28
	0.270	0.186	4.51	0.352	0.209	—
	0.302	0.210	2.95	—	—	—
25° C.	0.375	0.210	4.05	0.269	0.156	3.55
	0.280	0.164	—	0.249	0.162	3.67
	0.164	0.156	5.78	0.227	0.162	2.47
	0.357	0.189	5.14	—	—	—
30° C.	0.405	0.249	3.36	0.312	0.213	3.22
	0.313	0.233	6.50	0.315	0.264	4.56
	0.301	0.266	5.06	0.262	0.180	4.88
35° C.	0.383	0.275	5.79	0.319	0.241	3.45
	0.541	0.464	4.72	0.433	0.299	3.68
	0.883	0.359	8.70	0.319	0.255	4.60
40° C.	0.404	0.193	6.48	0.400	0.142	3.70
	0.491	0.289	3.99	0.362	0.116	5.19
	1.000	0.286	3.25	0.375	0.102	1.47
	0.902	0.261	2.57	0.317	0.036	2.32
	0.371	0.171	4.83	0.355	0.093	—
	0.580	0.422	4.32	—	—	—
	0.481	0.302	2.06	—	—	—
45° C.	0.985	0.147	Fungus	0.247	0.114	—
	0.632	0.177	visible	0.266	0.074	3.19
	0.623	0.150	next day	0.314	0.057	2.94

One other discrepancy must be noted. At 21° C. both lichens gave mean initial values higher than might be expected, and showed also a rather wide variation. These high values for each species were due to two determinations which, as it happens, were the earliest estimates made. It has not been possible to trace them to any definite error, but it seems likely that they may have been due to lack of experience of the method.

At temperatures of 40° and 45° C. the decrease in rate which occurs is very much more rapid than at lower temperatures, as can be seen from Table III. The respiration rate at the end of five hours at all temperatures from 10°–35° C. is of the order of 60–70 per cent. of the initial rate, and the nature of this decline has been discussed above. At 40° and 45° C., however, the final rate is only about 30 per cent. of the initial rate. This can only be due to the injurious action of the temperature on the respiratory activity. It may seem surprising that below 40° C. there is no indication of a time-effect of temperature, but this agrees very closely with the results obtained by Kuijper (22) for peas. His figures show that, though a slight time-effect is first seen at 25° C., a pronounced effect occurs only at 40° C.

Between 10° and 35° C. the initial respiration rate rises with temperature. Values for  $Q_{10}$  for various intervals are shown in Table III; they are calculated from first determinations only, and it will be seen that they are fairly constant at about 1.5. The value for 10°–15° C. is rather higher. The aberrant respiration value at 21° C. has not been employed in the calculation, the interval 15°–25° C. being used instead. The reliable values for *P. canina* are  $Q_{10} = 1.56$  for interval 10°–15°, and  $Q_{10} = 1.67$  for interval 15°–25° C. These agree closely with the longer series for *P. polydactyla*. This value seems to be rather low, but it is of the same order as that found by Kuijper for peas between 20°–35° C.; the higher values which he obtained at lower temperatures have not been observed.

TABLE III.  
*Respiration of P. polydactyla (Mean Values).*

Temperature.	Determinations		$\frac{B \times 100}{A}$		$Q_{10}$
	A.	B.			
10° C.	0.130	0.069	53	}	1.71
15° C.	0.170	0.102	60		1.44
21° C.	0.245	0.120	49		
25° C.	0.245	0.160	65		
30° C.	0.296	0.219	71	}	1.45
35° C.	0.357	0.265	74		1.45
40° C.	0.362	0.098	27		1.03
45° C.	0.284	0.081	29		

Only Stocker working on *Umbilicaria*, and Henrici, who used *Peltigera* and several other lichens, have previously investigated this coefficient.

Very few estimations were made by either of these investigators, and their results were irregular. Stocker's results, however, are of the same order, especially at lower temperatures, as those here set forth. A single series, given by Henrici, gives such high values that doubt must be expressed as to its accuracy. At zero a respiration rate of 1.2 mg. CO<sub>2</sub> per grm. fresh weight per hour was obtained, and this is four times the average value obtained at 35° C. in the present experiments.

Stocker obtained appreciable respiration at 50° C., and Henrici claims to have demonstrated it at -12° C. Extrapolation from the present results seems to indicate that respiration may take place several degrees below zero.

Comparison with the rates of respiration of the higher plants is perhaps hardly permissible, but it is of interest to note that the rate obtained at 10° C. in both species of *Peltigera* is of the same order as the average rate of a mature sunflower as determined by Briggs (9). The respiration rates of *P. polydactyla* and *P. canina* per grm. dry weight per hour at 10° C. are 0.52 mg. and 0.64 mg. respectively, while that of the sunflower, 85 days old, is 0.53 mg.

#### WATER CONTENT AND RESPIRATION.

Previous investigators have all found decrease of respiration with decreasing water content, with slight variation in the type of curve which will be discussed later. In the following experiments the two lichens *P. canina* and *P. polydactyla* were again used, and the work carried out at 15° C.

*Method.* The respiration of the saturated lichen over a period of one hour was in all cases determined first; the material was then removed, dried in air to approximately the desired degree, and replaced in the flask for a second determination. Water content was obtained by weighing the material before and after each determination and by determining dry weight after the experiment. When the lichen is saturated a slight loss of water takes place during the experimental hour; at lower water contents loss is negligible, and the water content during the experiment may be taken as constant. The values obtained are given in Table IV and are plotted in Fig. 5.

As usual the variation due to the use of different material is considerable, but there is no doubt that intensity of respiration is closely related to water content. Inspection of Fig. 5 seems to justify the conclusion that the relation is nearly linear. There is an indication that maximum respiration is reached at a water content between 80 per cent. and 90 per cent. of saturation; there is no evidence that increase of water content beyond this point is accompanied by a fall in respiration.

Stocker, Fraymouth, and Jumelle, who have previously investigated this relation, all found that maximum respiration occurred at a water

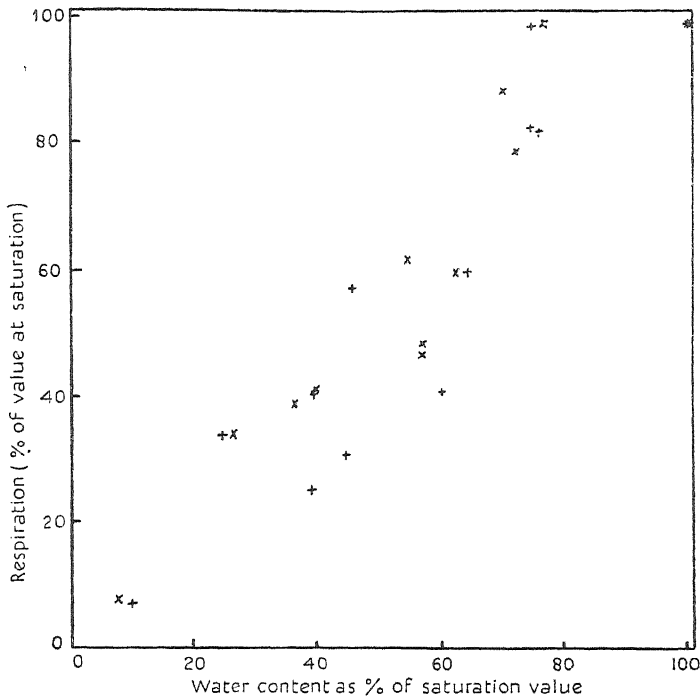


FIG. 5. Respiration and water content of *P. canina* (+) and *P. polydactyla* (x).

TABLE IV.

*Water Content and Respiration at 15° C.*

<i>P. canina</i> .		<i>P. polydactyla</i> .	
Water content (%).	Respiration (%).	Water content (%).	Respiration* (%).
74.6	82.6	77.3	100.7
74.5	98.5	71.6	79.2
75.6	81.5	68.9	89.4
63.9	59.8	62.0	59.5
59.8	41.7	56.7	46.2
45.4	30.8	56.5	48.2
44.5	57.1	53.6	62.2
40.0	40.5	40.3	40.6
39.0	25.6	36.1	39.0
25.4	32.5	27.2	32.5
9.9	6.5	6.7	6.6

content considerably below the saturation point, Stocker at 75 per cent. and Fraymouth at 50 per cent., and that further increase in water content led to a fall in respiration rate. It is possible that lichens with a less efficient aerating system than *Peltigera* may behave thus, and it may be

noted that Fraymouth's experiments were made with *Parmelia*. The presence of condensation moisture on the surface of the lichen might also lead to a decrease in respiration rate. Even in the air-dry lichen, with only 6.7 per cent. of the maximum water content, respiration is still demonstrable. A set of determinations carried out at 25° C. gave similar results. It is of interest to note that White (32) found 'slight' and 'extremely slight' respiration for stored seeds of *Acacia melanoxylon* and of *Pinus insignis* which contained 4.8 and 5.3 per cent. water respectively.

### III. ASSIMILATION.

For assimilation work it was necessary to modify the respiration apparatus in respect of leaf chamber, lighting, and CO<sub>2</sub> supply. A modification of F. F. Blackman's leaf-chamber method (3) was employed, and the arrangement for illuminating the plant is shown in Fig. 6.

#### *Plant Chamber and Lighting.*

The plant chamber was a flat rectangular glass box measuring 10 × 8 × 1 cm., with inlet and outlet tubes at opposite ends, and with a thermometer, the bulb of which lay below the lichen. The bottom and sides were blackened, and the top could be removed to insert the lichen. The top was fixed in position with vaseline and paraffin wax. The lamp was enclosed in a metal cylinder with a glass floor, and through this a current of water was run to avoid heating effects. Variation in light intensity was secured by raising and lowering the lamp, and by using different lamps. The enclosing cylinder rested on the plant chamber, and arrangements were made to exclude light from other sources. The light intensities for the different lamps used, and for various positions, were measured by a McBeth illuminometer, and are expressed as lux, i.e. metre-candles (Hefner).

#### *Carbon Dioxide Supply.*

The air current was enriched with carbon dioxide by F. F. Blackman's method, that is, by allowing dilute hydrochloric acid to drop from a Mariotte bottle aspirator on to marble chips contained in a Reiset tower. The rate was controlled by capillary tubing on the exit arm of the aspirator, to produce about 0.6 per cent. CO<sub>2</sub> in the current for the 'excess' supply: lower concentrations were obtained by diluting the acid. At high carbon dioxide contents the concentration was not always constant, but under these conditions slight variations in concentration did not affect assimilation rate.

The control branch of the air current passed through a U-tube of about 40 c.c. capacity on the way to the Pettenkofer tubes. The volumes of air passing through plant chamber and U-tube were measured by the



loss of water from the aspirators. The carbon dioxide content of the control branch current was used as the basis for calculating the amount of carbon dioxide absorbed in the plant chamber. In the series of experi-

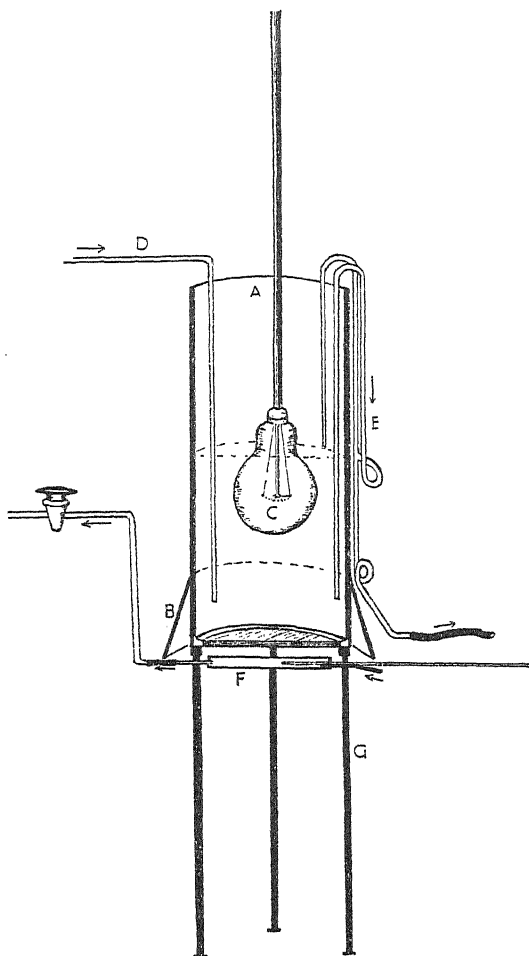


FIG. 6. Illumination apparatus for assimilation experiments. A, metal cylinder with glass bottom; B, cowl; C, lamp; D, entrance tube for cooling water; E, constant-level siphon; F, plant chamber; G, supporting tripod.

ments on the relation of assimilation to light intensity, water content, and temperature, where carbon dioxide was supplied in large excess, the content of the gas in the entrance current is also taken as the concentration in the chamber.

The accuracy of the division of the air current was tested by making a series of parallel blank determinations with ordinary air, and with air containing about 0.1 per cent. carbon dioxide. The difference between pairs of determinations did not exceed 3 per cent.

*Assimilation and Light Intensity.*

After various trials it was decided to make a series of estimations on two different plans. The preliminary treatment of the material was always the same. The lichen was kept in a cool greenhouse. In the morning a sample, freed from the moss in which it grew by cutting off the rhizoids, was rinsed in distilled water and dried by gentle pressure between filter papers. It was then weighed and placed in the chamber. The sample had an area of about 40 sq. cm., fresh weight about 1.5 grm., and dry weight about 0.4 grm. The remains of the rhizoids held the lichen sufficiently above the bottom of the chamber to allow free circulation of air. The air current was drawn through the chamber for seven minutes in the dark, a time sufficient for one change of the atmosphere, and then for ten minutes in the light. At the end of the period the Pettenkofer tubes were shunted into the air currents by a suitable arrangement of three-way stopcocks, and absorption allowed for half an hour. Before the next period an interval of seven minutes was allowed during which the air current was drawn at the same rate through the chamber. This interval allowed when necessary for the adjustment of a new light intensity, and for changing the Pettenkofer tubes. The somewhat complicated arrangement of taps was always manipulated in the same order. Precautions were taken to avoid access of air to the baryta at any point of the manipulations.

The carbon dioxide supply was about 0.6 per cent. (12 mg. per litre) throughout, a concentration well above the limiting value, as will be shown later. The temperature was 20° C. At this temperature the loss of water which always takes place, even in a current of saturated air, is not very great. At the higher light intensities the temperature of the chamber tended to rise, and this was counteracted by adding cold water to the bath. This adjustment could also be carried out in the seven-minute interval between absorptions.

Assimilation is expressed as mg. CO<sub>2</sub> absorbed per 1 grm. fresh weight per hour. As the weight decreases somewhat during the experiment, the mean of the initial and final values is used. Dry weight and area are also measured and results calculated in terms of these, but as no difference in behaviour is shown by these figures, they are not given.

The two different series of estimations referred to above were carried out as follows:

(i) Two half-hour estimations were made at a light intensity of 5,623 lux followed by two at a higher intensity. The results are given in Table V and plotted in Fig. 7 a.

(ii) One half-hour estimation was made at an intensity of 1,959 lux followed by single estimations at 5,623, 11,010, 22,880, and 44,920 lux.

The results of the six sets made on this plan are given in Table VI and plotted in Fig. 7 *b*.

The mean of all values obtained for each light intensity in both series are plotted in Fig. 8. A graph for *real* assimilation, obtained by adding to each value for *apparent* assimilation the standard respiration value of 0.25 mg., is also given in this figure.

TABLE V.  
*Assimilation and Light Intensity.*

Temp. 20° C.

Light intensity (lux).	Assimilation (mg. CO <sub>2</sub> per hour per grm. fresh wt.).							
	Dec. 13.	Dec. 17.	Jan. 9.	Jan. 10.	Jan. 15.	Jan. 16.	Jan. 20.	Jan. 21.
5,623	1.172	1.247	1.198	1.382	1.554	1.099	1.705	1.317
11,010	2.132	—	—	—	—	1.732	—	2.576
22,880	—	2.691	2.514	—	—	—	2.822	—
44,920	—	—	—	2.700	2.672	—	—	—

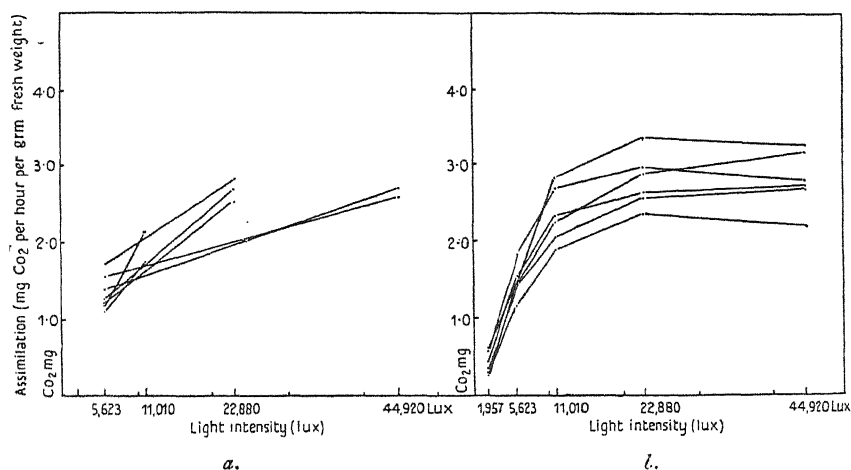


FIG. 7. Assimilation of *P. polydactyla* at various light intensities. (a) Each line joins the two values obtained in a single experiment. (b) Each graph represents the five determinations in a single experiment.

The course of the two graphs is very similar. It will be seen that, although there is variation between different samples, the graphs show a satisfactory agreement.

From these results we may obtain the following information :

(1) The values of real assimilation at 1,957 and 5,623 lux show that an approximate proportionality with the light intensity exists in this region.

TABLE VI.  
*Assimilation and Light Intensity.*

Temp. 20° C.

Light intensity (lux).	Assimilation (mg. CO <sub>2</sub> per hour per grm. fresh wt.)					
	Jan. 22.	Jan. 24.	Jan. 27.	Jan. 28.	Jan. 30.	Jan. 31.
1,957	0.61	0.42	0.32	0.23	0.26	0.57
5,623	1.47	1.54	1.43	1.48	1.16	1.82
11,010	2.24	2.32	2.05	2.82	1.89	2.68
22,880	2.89	2.62	2.55	3.34	2.33	2.96
44,920	3.14	2.72	2.66	3.23	2.20	2.77

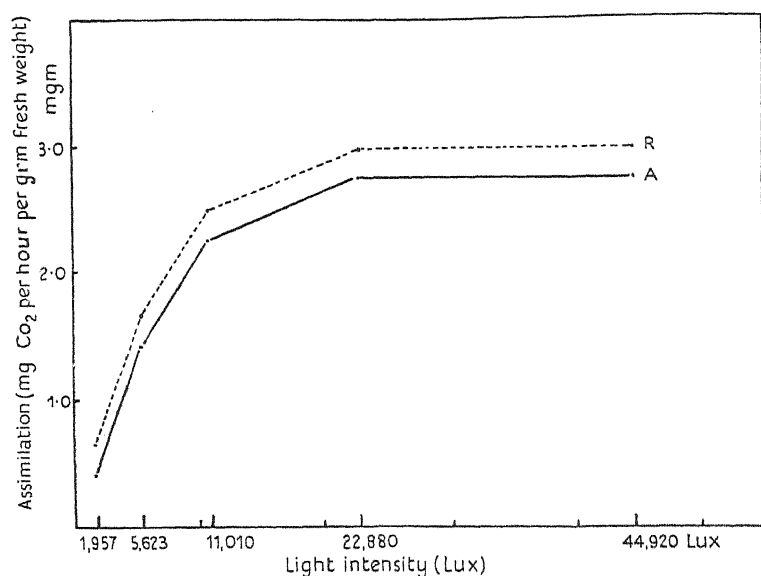


FIG. 8. Assimilation of *P. polydactyla* and light intensity. Each point is the mean of all the values for that light intensity. R, real assimilation; A, apparent assimilation.

(2) Between 5,623 and 22,880 lux an increase in assimilation rate again occurs, but with smaller increments as the intensity rises. The graphs up to this point are strikingly similar to those obtained by Warburg (31) for *Chlorella*.

(3) Above this point (22,880 lux) no further increase in assimilation is obtained. It will be shown in the next section that in this region assimilation is limited by temperature. At a higher temperature the diminishing rate of increase found by Warburg, resulting in a gradual flattening of the curve, would doubtless take place.

(4) The relation found agrees with F. F. Blackman's (4) conception of one factor controlling the reaction, in so far as the first and last portions of

the graph are concerned; it agrees, however, with the work of later investigators as to the course of the graph in regions of medium intensity.

Henrici carried out two sets of two determinations on *P. polydactyla*, the results of which are given in Table VII.

TABLE VII.

*Assimilation and Light* (Henrici: extract from Table III).

*P. polydactyla* CO<sub>2</sub> = 1.8 %.

Lux.	75.	100.	225.	400.	900.	2,000.	3,500.	8,000.	32,000.
Assim.	1.4	2.2	6.7	1.4	3.4	5.2	4.4	2.7	2.4
(mg./f. w. per hr.)	18.5	9.2	10.8	9.1	11.2	3.3 <sup>1</sup>	0	—	—

It will be seen that in both these experiments a double maximum was obtained, and that above the second maximum assimilation again decreased, reaching zero in one experiment at 3,500 lux. It may be noted that Boysen-Jensen found the compensation point, that is the light intensity at which respiration and assimilation balance, at 4,000 lux for 20° C. The course of my graphs indicates that there is a compensation point at about 500 lux. The high compensation point found by Boysen-Jensen may well be due to the short period (ten minutes) of his experiment, which would tend to make it impossible to measure very low values.

It is difficult to understand why an increase in assimilation should take place at still lower intensities. In view of the great regularity of the graphs shown in Fig. 7, and of the fact that none of them gives any indication of a decrease, even although the light intensities employed were higher than any of those used by Henrici, it must be suggested that the high light values she finds are due to experimental error. If the results are calculated for area instead of fresh weight, no significant change in the course of the curve is indicated.

#### IV. TEMPERATURE AND ASSIMILATION.

An increase in temperature has been shown to affect the rate of assimilation in higher plants somewhat in the same manner as it affects the rate of a monomolecular chemical reaction. The rate is approximately doubled for every increase of 10° C. Above a temperature of about 30° C. in assimilation experiments a second effect, the 'time factor' of Blackman, appears, and results in a progressive fall in rate at any constant temperature with time.

In the experiments here described the CO<sub>2</sub> supply maintained was high, 0.5 per cent. in all experiments. Determinations were carried out at

<sup>1</sup> This value was obtained on the second day.

two light intensities, high, 22,880 lux, and low, 5,600 lux. The highest light intensity available, 44,000 lux, was not employed on account of the rapid drying-out which takes place. A preliminary period of seven minutes

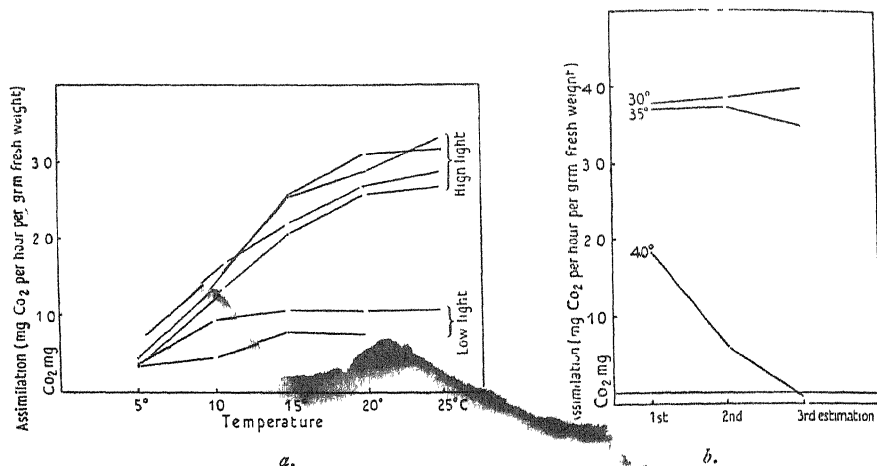


FIG. 9. Temperature and assimilation of *P. polydactyla*. (a) Groups I and III. (b) Group V, time factor.

in the dark preceded the first half-hour estimation in each experiment. Between estimations fifteen minutes was required to alter the temperature. During this period the lichen was darkened and kept in the usual air current. A further period of seven minutes in the light then preceded the next half-hour estimation.

Five groups of estimations were carried out as follows:

- Group I. High light. 4-5 different temperatures in rising sequence.
- II. High light. 3-4 different temperatures in varied sequence.
- III. Low light. 4-5 different temperatures in rising sequence.
- IV. High light. One temperature only.
- V. High light. Three successive estimations at each of three high temperatures.

The estimations in each group were repeated several times. The results are given in Table VIII and plotted in Fig. 9 *a* and *b* for Groups I, III, and V.

There is a steady increase in assimilation rate with a temperature rise from 5° to 20°, and a lower rate of increase to 30°.

$Q_{10}$  values have been calculated from the averages of all determinations in Groups I and II, and they are as follows:

Temperature	5°—10°	10°—15°	15°—20°	20°—25°	25°—30°
$Q_{10}$	3.09	3.06	1.62	1.2	1.23

These are of the same order as those found by Blackman and Matthaei (5) for the cherry laurel.  $Q_{10}$  at 10°—20° is 2.23 for the lichen and 2.12 for cherry laurel. The  $Q_{10}$  values, however, fall off more rapidly at

TABLE VIII.

### Temperature and Assimilation.

Assimilation (mg. CO<sub>2</sub>/hr. per gm. fresh wt.), Carbon dioxide conc. = 0.5 per cent.

Group I.			Group II.			Group III.			Group IV.			Group V.		
22,880 lux.			22,800 lux.			5,600 lux.			22,800 lux.			22,800 lux.		
Date.	Temp.	Assim.	Date.	Temp.	Assim.	Date.	Temp.	Assim.	Date.	Temp.	Assim.	Date.	Temp.	Assim.
° C.			° C.			° C.			° C.			° C.		
Feb. 5	11·5	1·54	Feb. 12	5	0·68	Feb. 5	5	0·32	Feb. 26	30	2·67	Mar. 3	30	3·97
	15	2·55		20	3·15		10	0·42		35	2·75		30	3·84
	20	3·10		15	2·30		15	0·80		20	2·44		30	4·18 ?
	25	3·14	Feb. 13	20	3·12		20	0·71	Feb. 27	25	3·14	Mar. 14	30	3·58
Feb. 6	6·5	0·45		15	2·21		25	0·42	Feb. 28	10	1·02		30	3·86
	11·7	1·34		10	1·30	Feb. 7	5	0·35		15	1·86		30	3·87
	15	2·04		5	1·15		10	0·94		20	2·35	Mar. 28	35	4·01
	20	2·58	Feb. 17	10	1·12		15	1·07	Mar. 1	5	0·32		35	4·20
	25	2·69		5	0·78		20	1·05	Mar. 4	40	2·32		35	3·60
Feb. 7	6	0·74		10	1·34		25	1·07		45	0·51	Mar. 31	40	2·17
	12	1·67		15	2·74								40	0·82
	15	2·19	Feb. 18	5	0·64								40	+0·13
	20	2·69		10	1·39							Apr. 3	35	3·45
	25	2·86		15	2·43								35	3·29
Feb. 11	5	0·44	Feb. 19	10	1·20								35	3·29
	10	1·32		20	3·08							Apr. 4	40	1·47
	15	2·53											40	0·51
	20	2·87											40	0·01
	25	3·33											40	0·01

higher temperatures than do those of Blackman. Warburg found for *Chorella* a temperature coefficient falling off from 4.7 for 5°–10° to 1.6 for 20°–30° C. This agrees with the results here put forward, which are also those for an alga. It is clear, however, that the primary temperature relation of the lichen gonidia is the same as that of a phanerogam. The falling off in the  $Q_{10}$  at 25° and 30° C. might be explained as due to loss of water at these temperatures, but since the same result was obtained by Warburg for an alga in water culture it is more likely to be a primary internal effect.

It might be thought that the lower rate of increase between 20° and 25° C. was due to light at 22,000 lux being limiting. As, however, a further increase between 25° and 30° takes place this cannot be so, and it is safe to assume that the values obtained with 22,000 lux at 25° C. and probably at 30° C. are limited by temperature and not by light.

In Group III (Fig. 9 *a*) at low light the curve follows a similar course to that at high light from 5°–10°, when the limiting effect of light becomes very evident, preventing any rise after 15° C. The first experiment here gave low and rather irregular results, which might be attributed to poor material, but they are not at variance with any conclusions drawn.

To avoid the loss of water which takes place during a prolonged experiment a set (Group IV) was used, employing fresh material for each temperature, only one determination being made. The results (though only based on one estimation at each temperature) show that the curve is approximately the same as those of Groups I and II up to 25° C. At 30° C. there is a drop in assimilation, but the experiments of Group V show that this estimation and that at 35° C. are rather below the average. There is a very marked drop at 40° C. and 45° C., also confirmed in Group V.

The last group (V) is a study of the 'time-effect' at higher temperatures; three half-hour estimations were made over a period of about four hours, with the usual intervals of darkness. The values at 30° C. show no signs of falling off during the four hours. At 35° C. a slight fall occurs, but at 40° C. a very definite decrease to the compensation point is evident. Thus it appears that the injurious effect of temperature begins between 35° C. and 40° C. This is markedly higher than the temperature at which the time-effect sets in for cherry laurel, where the rate of falling off at 30° C. is much the same as that for the lichen at 40° C.

Comparison with the other investigations on lichens may now be made. Only Stocker (29) and Henrici (17) have worked in this field. Henrici, for *Peltigera*, gives a rise in assimilation up to 15° C. only, and for other lichens finds a variety of curves showing maxima at different temperatures, or none at all. Stocker's observations are more regular. The results on the temperature effect given here appear to be definitely at variance with his work, though his experiments, too, were carried out on



foliaceous lichens, with series of temperatures and short preliminary periods. He finds the peculiar double maximum curve; the same curve occurs in all the light intensities with which he worked, and for both lichens. He gives three curves, two for *Umbilicaria* and one for *Lobaria*, showing a maximum at about 10° C., a minimum about 20° C., and a second maximum about 25° C., the compensation point being reached at 35° C. He observes that the second maximum is not so high as the first, the highest rate being about 2.7 mg. for 100 sq. cm., or 1.08 for 40 sq. cm., the area of the fresh material generally used in my experiments. Secondly, he finds a minimum respiration at the minimum of assimilation (in one curve) which would support his view of the validity of the latter.

In considering the validity and significance of Stocker's two maxima the following observations may be relevant: (1) The fact that his second maximum value is lower than the first in spite of the higher temperature, and that the curve on the whole shows a tendency to decrease from the value at 10° C. right to the compensation point at 35° C. suggests a 'time-effect'. (2) This is the more likely if it is remembered that in long experiments loss of water always occurs, which tends to decrease the assimilation rate. Stocker makes as many as seven serial estimations, and it is not stated that the lichens were saturated with water between each. (3) The actual values observed are small, so that such variations as occur in a series of estimations would diminish the significance of the maxima and minima. (4) The periods of estimation were from fifteen to thirty-five minutes only, which tends to increase the error. (5) Most of the above variations or sources of error occur in each experiment, which may explain the similarity of all his curves.

These facts all seem to point to the conclusion that Stocker's double maximum is either not significant, or that his graphs really represent a variety of effects and not the simple relations between temperature and assimilation.

We may therefore conclude that a steady increase in assimilation with rise of temperature up to the point at which the injurious effects of heat appear (approximately 35° C.), with consequent fall in the initial values and ensuing time-effect, represents the temperature-assimilation relation of lichens and higher plants, rather than the double maximum which Stocker has observed in lichens, and Lundegårdh (24), with others, in higher plants.

A comparison may be made here between the assimilation of the lichen and that of the higher plants, using the maximum assimilation rates obtained for phanerogams by other workers.

The mean values obtained for temperature-limited assimilation for *P. polydactyla* are:

5°	10°	15°	20°	25°	30° C.
0.72	1.26	2.21	2.82	3.07	3.4

For higher plants the following values have been obtained :

<i>Helianthus tuberosus</i>	30° C.	27.3	Blackman & Matthaei.
" <i>annuus</i>	25° C.	23.0	Willstätter & Stoll (33)
<i>Prunus Laurocerasus</i>	29.5° C.	9.7	Blackman & Matthaei
" "	30° C.	9.9	Willstätter & Stoll

Both sets of values are calculated in milligrammes  $\text{CO}_2$ /hour/per gramme fresh weight. The assimilation rate for the phanerogams is shown to be of a very much higher order, though the respiration rates, as compared previously with the results of Briggs for a mature sunflower, appear to be of a similar order. The value of such a comparison should not be overestimated, but the lower assimilation in *Peltigera* would seem to agree with the slow rate of growth characteristic of all lichens. It is certainly to be related to the comparatively small proportion of assimilating cells in the lichen thallus. That the respiration rate is often of the same order in lichen and phanerogams is not strange since in respiration the much bulkier fungus symbiont is also active.

#### *Assimilation and Carbon Dioxide Concentration.*

The effect of carbon dioxide concentration on the assimilation of lichens has not been previously studied. Stocker assumed a direct proportionality of effect over the small range of variation in atmospheric carbon dioxide with which he worked. Much work has, however, been done on higher plants on this relation, notably by Blackman and Smith (6), Harder (16), Warburg (31), James (19), Boysen Jensen (7), and, much earlier, Brown and Escombe (10).

F. F. Blackman regards this relation of assimilation to carbon dioxide concentration as linear, an increase in carbon dioxide supply bringing about a proportional increase in assimilation, the graph representing this relation being a straight line. When, with increasing carbon dioxide supply, some other factor, for example, light, becomes limiting, no further increase in assimilation rate takes place when the carbon dioxide supply is raised. At this point the graph bends sharply and runs parallel to the axis of the abscissae.

Later observers, Harder and Warburg for water plants, and Lundegårdh (24) and Boysen-Jensen for land plants, obtained results which did not fit the theory of the single limiting factor. They found that (1) the graph relating assimilation to  $\text{CO}_2$  was curved to the axis of the abscissae, and not a straight line; (2) that for every combination of light and  $\text{CO}_2$  a rise of assimilation could be obtained by increasing either factor, so that a series of curves, each rising more steeply, was produced; and (3) they also state that the action of all the factors is not the same, but that the influence of the factor in relative minimum is the greatest. According to

Harder, the graph relating assimilation to  $\text{CO}_2$  supply consists of two main branches. With  $\text{CO}_2$  low, an increase in this factor has a large effect, the relation being nearly linear; where  $\text{CO}_2$  is high, light is relatively in minimum, and an increase in  $\text{CO}_2$  concentration has only a small effect. The two branches of the graphs do not meet at a sharp angle but through a curved portion, indicating a region where both  $\text{CO}_2$  and light have considerable effect.

Maskell (26) agrees that more than one factor may affect the process at the same time, and devises a series of graphs to show how results like those of Harder may be produced by a consideration of the effects of the various resistances to the diffusion of  $\text{CO}_2$ , and the interaction between light and  $\text{CO}_2$  at the chloroplast surface. The explanation given by Warburg depends on his theory of the nature of the photosynthetic chemical changes. Maskell sums up the situation by suggesting 'that the diverse types of interrelationship exhibited by the results of Blackman and Smith for *Elodea*, and of Harder and Warburg for water plants, may all be particular cases of a more general scheme'.

The particular interest of the lichen arises from the fact that it is a land plant in which the complication introduced by stomatal movement does not exist, that is, there is no stomatal resistance to the diffusion of  $\text{CO}_2$ . The assimilating cells lie in a loose network of hyphae separated from the atmosphere only by interhyphal moisture on the under surface, though the upper surface is more compact.

In the following experiments the effect of various  $\text{CO}_2$  concentrations was investigated and the relation of the supply through upper and lower surfaces. Two light intensities and two temperatures only were used, so that no detailed series similar to that of Lundegårdh (24) could be obtained. The effects produced, however, seem quite definite.

#### *Experimental Work.*

A fairly wide range of carbon dioxide concentrations was used, from 0.6 mg. to 16 mg. per litre. The different concentrations were obtained by changing the strength of the acid dropping into the carbonate tower, concentrations N/300, N/150, N/75, N/50, N/38, N/25 being used. Laboratory air provided the lowest concentrations.

In each experiment five estimations of half an hour were made in various concentrations of  $\text{CO}_2$ . A temperature of  $20^\circ\text{C}$ . was adopted, as for the light experiments, and illumination of 22,880 lux for one set of experiments was followed by a set at low light intensity, 5,600 lux. Two further experiments were also carried out at a temperature of  $25^\circ\text{C}$ ., one for each of the two light intensities.

The results are given in Table IX. Graphs for each experiment at  $20^\circ\text{C}$ . are shown in Fig. 10 *a*, and two composite graphs, one for high

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light and one for low are plotted in Fig. 10*b*. The concentration of CO<sub>2</sub>

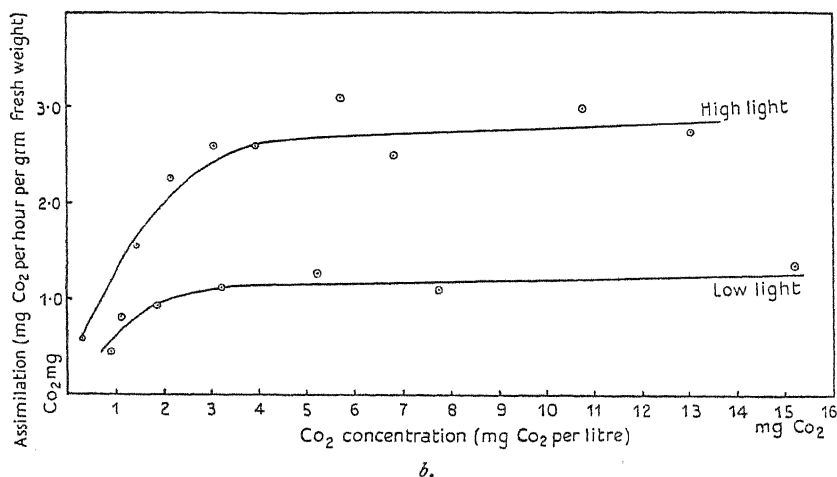
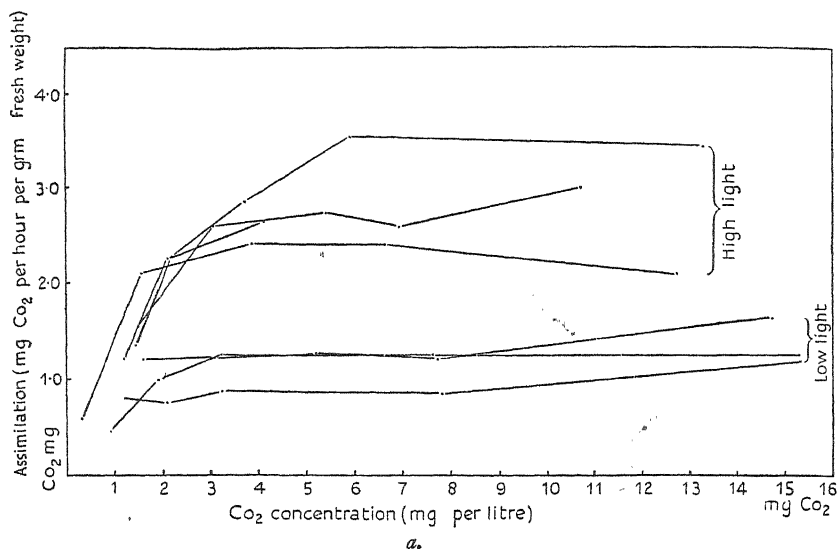


FIG. 10. Carbon dioxide supply and assimilation of *P. polydactyla*. (a) Graphs of individual experiments. (b) Composite graph for all experiments.

in the chamber is not taken as that entering but is calculated from the formula.

$$C = \frac{C_1 - C_2}{\log_e (C_1/C_2)},$$

where  $C_1$  and  $C_2$  are concentrations before entering and after leaving the leaf chambers respectively.

The values for one twenty-minute period experiment at high light, and 20° C. are unusually high, but the course of the graph corresponds to

that of the others, and the difference must be referred to material. The series at 25° C. shows higher values than those for the other three series in high light, and indicate that these are temperature-limited.

TABLE IX.

*Assimilation of P. polydactyla, and Carbon Dioxide Concentration.*

Assimilation is given as mg. CO<sub>2</sub> per grm. fresh wt. per hour; carbon dioxide concentration in mg. per litre.

	Assim.	CO <sub>2</sub> conc.	Assim.	CO <sub>2</sub> conc.	Assim.	CO <sub>2</sub> conc.	Assim.	CO <sub>2</sub> conc.
(a) High	April 14.		April 15.		April 16.		April 17.	
Light	1.21	1.23	1.36	1.44	1.55	1.47	0.57	0.27
22,880 lux	2.24	2.12	2.28	2.20	2.59	3.05	2.09	1.57
Temp. 20° C.	2.63	4.19	2.85	3.78	2.75	5.53	2.42	3.97
	—	—	3.54	6.01	2.59	7.04	2.40	6.72
	—	—	3.44	13.39	3.02	10.82	2.08	12.85
(b) Low	April 23.		April 27.		April 29.			
Light	1.20	1.63	0.80	1.15	0.42	0.94		
5,000 lux	1.23	3.12	0.75	2.07	1.00	1.97		
Temp. 20° C.	1.27	5.28	0.88	3.32	1.27	3.26		
	1.21	7.84	0.83	7.89	1.26	7.71		
	1.63	14.84	1.21	15.39	1.25	15.37		
(c) High	April 30.		Low	April 25				
Light	1.88	1.28	Light	0.34	0.49			
Temp. 25° C.	3.11	4.36	Temp. 25° C.	0.90	1.17			
	3.29	6.67		1.12	5.13			
	3.10	14.07		0.94	8.21			
				1.41	16.31			

*Results.*

The relation shown by the graphs of single experiments is the same throughout, the tendency being shown more clearly in the composite graphs. At low CO<sub>2</sub> concentrations there is a linear relation between CO<sub>2</sub> and assimilation rate; at high CO<sub>2</sub> concentrations the graph is parallel to the axis of the abscissae, and here light is clearly limiting. As there is no sharp inflection in either, there is a region in which light and carbon dioxide concentration both affect the assimilation rates. There is an indication that the high-light graph lies above the low-light at low concentration, in agreement with the observations of Lundegårdh (24), but the number of estimations is too small to allow a definite conclusion to be drawn.

The graphs illustrate the limiting effect of carbon dioxide in low concentrations and of light or temperature at the high concentrations, but there is undoubtedly an intermediate range not explained by the action of a single limiting factor.

*Water Content and Assimilation.*

It is generally accepted that in both the higher and lower plants decrease in water content lowers the rate of assimilation as well as that of respiration. Dastur (12) and Plantefol (27) found a rough proportionality in phanerogams and mosses, though employing widely different methods of estimation. Walter (30), using water plants immersed in sugar solution of different strengths, also found that the assimilation intensity is proportional to the water content of the cell-sap which he supposes to affect the water content of the plasma. Jumelle finds a similar relation for lichens, with maximum assimilation rate slightly below the maximum water content. Stocker's results agree with Jumelle's, but his assimilation maximum occurs at a lower water content.

*Method.*

Five estimations on the same lichen were completed during one day, covering the whole range of water content, the lichen being partially dried out between the estimations. Single half-hour estimations were made for each water content, and 'high' light (22,880 lux) and high CO<sub>2</sub> (12 mg. per litre) were used, with a temperature of 20° C. Four sets were carried out in this way, and the results are given in Table X and Fig. 11. The assimilation is calculated as mg. CO<sub>2</sub> per grm. of the original fresh weight.

TABLE X.

*Assimilation of P. polydactyla and Water Content.*

Light 22,800 lux., temp. 20° C., carbon dioxide 12 mg. per litre.

Date.	Assimilation (percentage of value at saturation).	Water content (percentage of valuation at saturation).
March 10	100.0	100.0
	26.4	50.2
	-6.1	22.7
March 12	100.0	100.0
	100.5	82.4
	43.4	55.2
	11.5	33.1
March 17	11.5	19.6
	100.0	100.0
	89.6	72.5
	55.5	60.3
	2.9	33.2
March 19	-0.5	16.9
	100.0	100.0
	75.0	74.5
	58.4	49.4
	15.6	30.2
	-11.5	17.1

Fig. 11 shows that the results are more regular than those for respira-

tion, and clearly indicate a linear relation between water content and apparent assimilation. With increasing water content the maximum assimilation rate seems to be reached between 80 per cent. and 90 per cent. of the

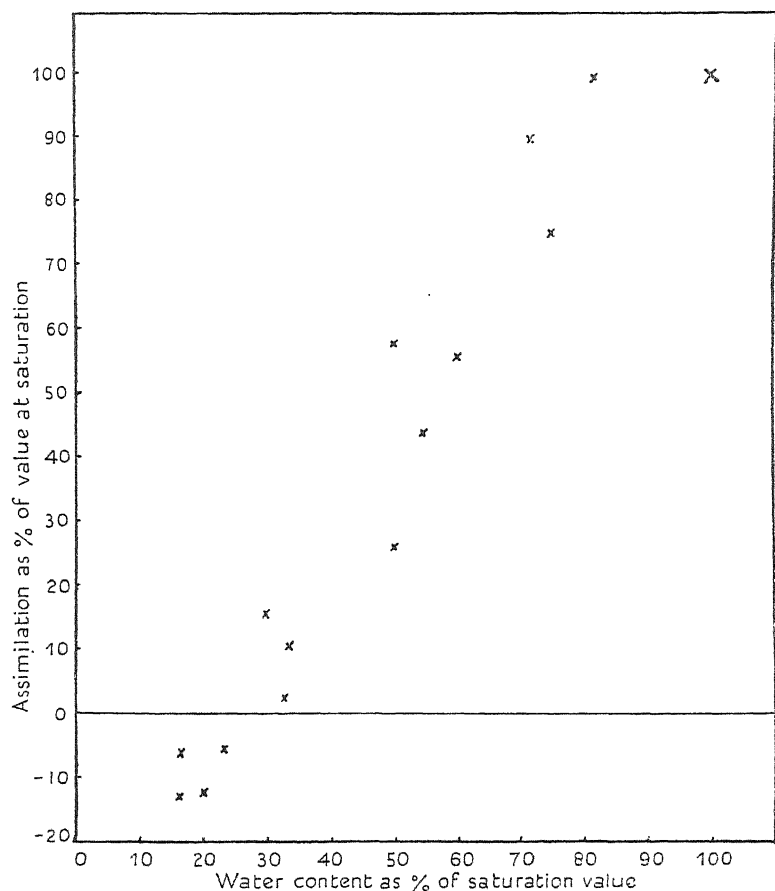


FIG. 11. Water content and assimilation of *P. polydactyla*.

maximum water content, at about the same value, that is, as the maximum respiration rate. Here again there is no indication of a decline at higher water contents. Assimilation falls off more rapidly than respiration, with the result that below 30 per cent. saturation respiration is more active, and evolution of carbon dioxide takes place even under favourable conditions of light, temperature, and carbon dioxide supply.

*The Permeability to Carbon Dioxide of the Upper and Lower Surfaces of the Lichen.*

A number of experiments were carried out to determine whether there is any difference between the rate of supply of  $\text{CO}_2$  through the upper and lower surfaces of the lichen.

*Method.*

It is not possible to compare the absorption of upper and lower surface of the lichen simultaneously, as Blackman succeeded in doing for phanerogams, because of the small area and irregularity of the lichen thallus. For each experiment, therefore, a sample of about six lobes of thallus was chosen, and each piece cut in half. The assimilation of the two sets of half-pieces thus produced was estimated separately by the usual method of the single half-hour period exposure to light. (light intensity, 22,880 lux; temperature 20° C.) The lower surfaces of one set were then vaselined and an estimation made of the assimilation in this condition, followed by a similar estimation of the second set, with the upper surfaces vaselined. In order to vaseline the under surface satisfactorily the rhizoids had to be cut off completely. The pieces were therefore laid across glass rods in the leaf chamber to ensure circulation of the gas beneath as efficiently as above them.

TABLE XI.  
*Carbon Dioxide Supply through Upper and Lower Surfaces of*  
*P. polydactyla.*

Light 22,800 lux., temp. 20° C.			
High CO <sub>2</sub> = 12.23 mg. per litre; low CO <sub>2</sub> = 3.37 mg. per litre.			
Date and CO <sub>2</sub>	Surface.	Assimilation in mg. CO <sub>2</sub> /hr./gram. fresh weight.	Assimilation as percentage of both free.
May 19	Both free	2.86	100
	Upper free	2.68	93.8
High CO <sub>2</sub>	Both free	2.88	100
	Lower free	2.71	94.1
May 20	Both free	2.76	100
	Upper free	2.23	80.7
High CO <sub>2</sub>	Both free	2.44	100
	Lower free	2.07	84.8
May 23	Both free	1.97	100
	Upper free	1.96	99.6
High CO <sub>2</sub>	Both free	2.06	100
	Lower free	1.82	88.2
May 21	Both free	1.78	100
	Upper free	0.90	50.4
Low CO <sub>2</sub>	Both free	1.73	100
	Lower free	1.66	95.9
May 22	Both free	2.00	100
	Upper free	0.97	48.6
Low CO <sub>2</sub>	Both free	1.87	100
	Lower free	1.88	100.5

In this way the closest possible approximation to estimations on the same lichen was obtained. The results are given in Table XI for three such experiments in high CO<sub>2</sub> concentration (0.6 per cent. by volume), and two



experiments in low  $\text{CO}_2$  concentration (0.2 per cent. by volume), which has been found to be limiting in the light intensity used. The low-concentration estimations were included because it was thought that the efficiency of the absorption by the single surface might not be the same in all concentrations of the gas.

With  $\text{CO}_2$  in excess the lichen with free upper surface assimilates on an average 91.4 per cent. of the rate of that with both surfaces free, with the lower surface 92.4 per cent.; the two surfaces are equally effective. With low  $\text{CO}_2$  the figures are 49.5 per cent. and 98 per cent.; the upper surface is thus half as effective as the lower.

This shows that under normal conditions the supply through the lower surface is much more efficient, and this will be the more important in nature as the lower surface is in close contact with the soil, moss, or dead organic matter, and, as Feher (13) has shown, is probably bathed in an atmosphere relatively rich in carbon dioxide.

Through the lower surface carbon dioxide supply takes place by gaseous diffusion right to the gonidial layer, while through the upper surface diffusion must take place through a liquid phase several cells deep. In high concentration of carbon dioxide diffusion in the liquid phase is evidently sufficiently rapid to maintain the maximum concentration necessary in the assimilating cells, but in low concentration this is not possible, and the advantage of the more rapid diffusion in the gaseous phase becomes apparent.

#### ECOLOGICAL OBSERVATIONS.

##### *Environmental Factors and Water Content.*

In August, September, and October 1929 a series of observations was made in the field with the object of gaining some knowledge of the manner in which different factors control the activity, especially the photosynthetic activity of the lichen in nature. An area, measuring about 20 yds. by 5 yds., was chosen in a clearing of an ash-oak wood in Cleeve Combe, Somerset. The area consisted of a sloping bank on the north side of the combe. One part was quite open, and the lichens grew on a substratum of loose stones which were often covered by a thin layer of moss. Another part was shaded by a fir-tree, and the remainder consisted of moss-covered soil sheltered in places by shrubby or herbaceous plants. In this area six different stations were selected, covering the range from complete exposure to complete shadow, and from each collections of lichen for determinations were made. The lichens were placed in stoppered bottles and the moisture content determined subsequently in the laboratory.

Collections were made at 11.30 a.m., 2.30 p.m., and 5.30 p.m. on five days selected to give a variety of meteorological conditions, the nature of which is shown in Table XII. Periodical records were made of temperature

in the sun, light intensity, and atmospheric humidity. Light was measured by a Bee meter (cf. Bracher (8)) and humidity by a wet and dry bulb thermometer.

TABLE XII.

*Weather Conditions during Ecological Observations.*

Date of observations.	Weather on previous day.	Weather on same day before experiment.	Weather during experiment.	Temperature in open.	
				Max. ° C.	Min. ° C.
October 2	Heavy rain in night	Steady rain till 9 a.m.	Cloudy, a little sun, shower at 1.25-2 p.m.	17	14
August 23	Rainy	Some rain before 8 a.m.	No rain, cloudy with some sun	25.5	17.2
August 29	A few thunder showers	No rain but woods still damp	Sunny till 4 p.m. then dull	44 (in the sun)	17.0
„ 26	No rain	No rain, mist before 8 a.m.	No rain, sunny	41 (in the sun)	20.2
September 9	Drought for a week	No rain, mist before 8 a.m.	Sunny, light clouds after 3.30 p.m.	45 (in the sun)	19.0

The data are plotted in Fig. 12. In addition to relative humidity the saturation deficit (saturation vapour pressure, less observed vapour pressure) is given. Light intensity is plotted in arbitrary units; direct sunlight at noon is taken as twenty. Each vertical line in the bottom section of the diagram represents the water content of one sample of *P. canina*, the six lines in a group corresponding to the six stations. The sample from the most sheltered station is on the left hand, that from the most exposed on the right. The difference in water content between the different stations of each collection is that shown by the lines of a single group: the difference in water content at different times and dates is shown by the different groups. Figures were also obtained for *P. polydactyla*, but as they give no additional information they have not been plotted.

It will be seen that it is only for a short time after heavy rain that the lichen is saturated with water. On October 2, with rain until 9 a.m., water content of 286 per cent. and 291 per cent. was reached. On August 23, when rain ceased before 8 a.m., the values are appreciably lower. On the latter day, though there was little sun, the water content fell off during the day, especially in the more exposed stations. Even on October 2, with low temperature and showery weather, a definite fall took place, if one

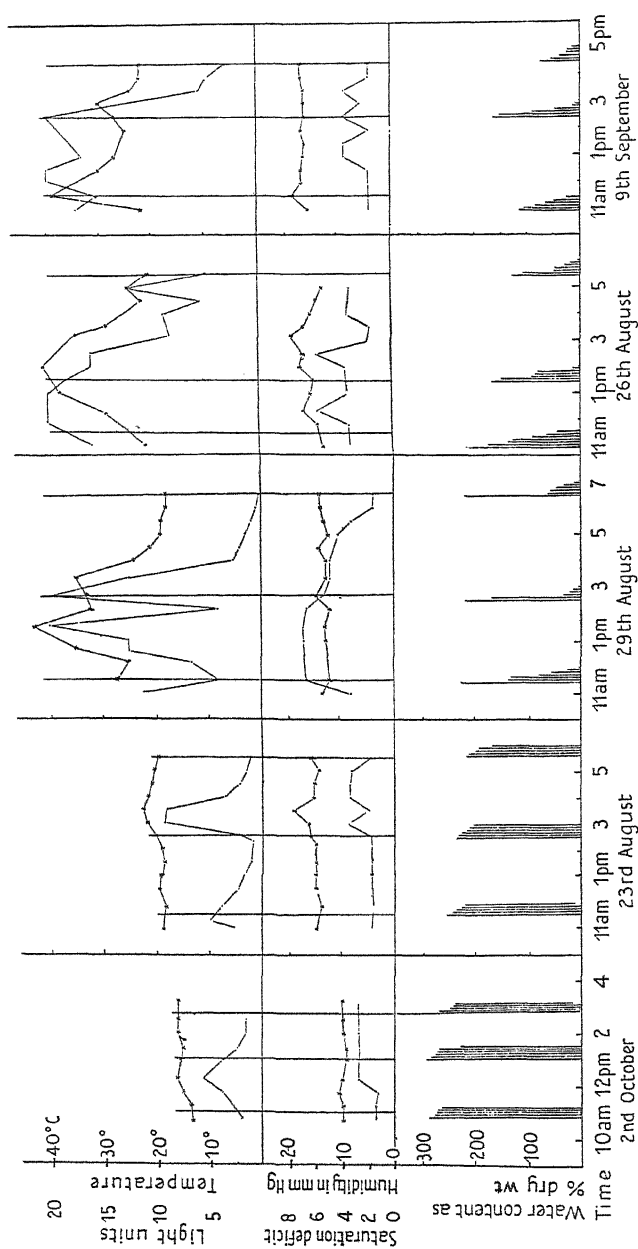


FIG. 12. Water content of *P. canina* and environmental conditions. Upper section, temperature (— x — x) and light (---). Middle section, humidity (— x — x) and saturation deficit (---). Lower section, water content of lichen; each vertical line represents the value for one sample.

sample with an abnormally high water content is disregarded. In the more exposed stations the water content is always lower than in the more shaded, but it should be noted that the first sample was collected rather late, at 11.30 a.m.

The collections of August 29 show the advantage to the lichen of the shaded position, the most shaded lichen retaining a good supply of water throughout a sunny day after being wet the previous night. In exposed stations the water falls to minimal values of about 10 per cent. The collections of August 26 and September 9 were made under drought conditions. Both days were preceded by misty nights, and on August 26 an initial water content of over 200 per cent. was recorded. This is more than the lichens absorb from a saturated atmosphere, and it may be assumed that mist, like dew, is an efficient source of supply. After a week of drought, however, the highest water content towards noon is only about one-third of saturation value, and exposed lichens are nearly air-dry in the afternoon.

#### *The Factor Controlling Photosynthesis.*

Although it is now known that the theory of limiting factors does not give a complete account of the relations of external factors to photosynthesis, it is still true that in nature that process is often limited by one factor.

From the experiments on the carbon dioxide relation it can be gathered that with  $\text{CO}_2$  at 0.04 per cent. (0.8 mg. per litre), a value of the order commonly found near the soil in woods, assimilation takes place at the rate of about 1 mg. per grm. fresh weight per hour at a temperature of 20° C., while at 25° C. the value is about 1.4 mg., and at 15° C. it is 0.7 mg. The rate at 20° C. requires a light intensity of 3,500 lux. During fine weather in summer light intensity is as low as this only late in the evening. On August 29 such an intensity was reached after 5 p.m.; on the two other fine days not until after 7 p.m. On those fine days the temperature in the sun was always over 20° C. In such weather, therefore, we may safely conclude that so far as external factors are concerned the concentration of carbon dioxide in the atmosphere limits the rate of assimilation. On the two days of wet weather, when the sky was overcast, there were considerable periods in the middle of the day when light intensity fell to about this value or rather lower. As, however, the temperature was also lower, fluctuating on October 2 about 15° C., the maximum possible assimilation must also have been somewhat low. In wet and cloudy weather in summer it is therefore likely that assimilation takes place at a rate which is approximately maximal, for both the light and the carbon dioxide available, sometimes the one and sometimes the other, will limit the rate.

Turning to the question of water content, we find from Table X that at 20° C. an assimilation rate of 1 mg. CO<sub>2</sub> per grm. fresh weight per hour can be reached only when the lichen contains about 50 per cent. of its maximum water capacity, or about 150 per cent. water. During the wet weather period this value was maintained throughout the day, but during the dry weather only the most sheltered sample had so high a water content; for after rain on the previous day most samples contained much less. After a day's drought no sample held so much water after noon. Most samples in dry weather retain less than 30 per cent. water throughout the greater part of the day, and this is the content below which respiration is more active than assimilation. Though no data are available for the early morning hours, it is very probable that higher water contents prevail then, and, in fine weather, it can only be in the early morning that a balance of assimilation to <sup>net</sup> respiration obtains for *Peltigera*.

It is, of course, of the fact that in discussing the ecological relations no great exactitude is possible, but it seems legitimate to draw the conclusion that the most favourable condition for assimilation of *Peltigera* in summer is dull, moist, or actually wet weather, when the lichen can function to the maximum capacity allowed by the prevailing concentration of carbon dioxide and of light. In fine weather a partial recovery of water content during the night may allow an assimilation balance to occur in the early morning; during the major portion of the day, however, lack of water will either limit assimilation or cause a deficit through respiratory excess.

## SUMMARY.

### I. *Water Relations.*

The graph expressing the rate of drying out of *Peltigera* is similar in form to that for an agar gel.

The lower surface of the *Peltigera* thallus loses water more rapidly than the upper; this is due to the greater evaporating area of the lower surface, and not to the presence of any protective structure on the upper surface.

The lichen absorbs water from a saturated atmosphere, but only 71 per cent. of the saturation water content is thus reached.

An air-dried lichen retains about 5 per cent. of the saturation water content; if dried over calcium chloride, 3-4 per cent.

### II. *Respiration.*

The rate of respiration between 10° C. and 30° C. increases with the temperature.

The temperature coefficient for 10° C. is about 1.5. Even at lower temperatures a falling off in rate occurs during an experiment lasting five

hours. This may be due to loss of water or to diminution of food substances.

At 45° C. the initial rate is less than at 30° C., and a very rapid falling off, due to a 'time-effect', occurs.

The rate of respiration increases with water content up to the saturation point. The relation of rate to water content is approximately linear.

### III. *Assimilation.*

The rate of assimilation increases with light up to about 22,000 lux, with the compensation point approximately at 500 lux (temperatures 20° C. and 0.6 per cent. CO<sub>2</sub> concentration) when the temperature becomes limiting. The falling off in the rate of increase of assimilation with light is gradual, giving a curve similar to that obtained by Warburg for *Chlorella*.

The assimilation rate increases with temperature between 10° C. and 35° C. The temperature coefficient for 10° C. is 3.06 between 10° C. and 15° C. and falls to 1.23 between 25° C. and 30° C., and is approximately unity between 30° C. and 35° C.

A time-effect first becomes evident at 35° C., and is very marked at 40° C. and 45° C.

No evidence of a double maximum, such as has been observed by other workers, was obtained.

Assimilation rate increases with rising water content, the relation being approximately linear. Maximum assimilation rate occurs at saturation water content and no evidence of a maximum at a lower water content, such as was observed by Stocker, has been obtained.

Data were obtained for the relation of assimilation rate to carbon dioxide concentration at two light intensities. At high light intensity the relation corresponds to that found by Warburg for *Chlorella*. At low light intensities there is a definite light-limited effect. The graphs expressing these relations are compared with those constructed by Maskell for the cherry laurel leaf.

In low carbon dioxide concentrations, carbon dioxide supply through the upper surface, as measured by assimilation, is only 50 per cent. of that through the lower; in high concentrations the supply through either surface is sufficient for an assimilation rate approximately 90 per cent. of that when both surfaces are free.

### IV. *Ecological Observations.*

In nature maximum water content of *Peltigera* is reached only after heavy rain.

Even after heavy rain with a saturated substratum, water content falls during the day, the rhizoids not maintaining an adequate water supply.

In showery weather lichens in sheltered positions maintain a high water content; in the open, water loss is rapid and severe. Early morning mists and dew are important sources of water supply.

The water content in dry weather may fall to 5 per cent. of the saturation value.

Carbon dioxide assimilation in nature must usually be limited by carbon dioxide supply or by water content of the lichens. It is only after heavy rain that the lichen can for a brief period develop its full assimilation capacity.

I should like to express my thanks to Professor O. V. Darbishire for suggesting this problem, and to Dr. Macgregor Skene for all the helpful advice and criticism which he has given throughout the course of the work. I am also indebted to Mr. M. G. Bennett of the Physics Department for the determination of the values of the light intensities employed in the assimilation experiments. My thanks are also due to the Colston Research Society for a grant defraying part of the expense of the work.

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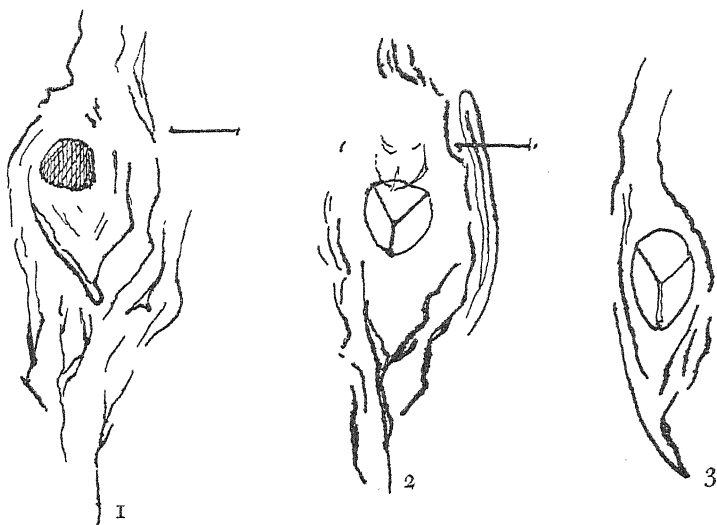
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## NOTES.

### NOTE ON THE 'SPORES' OF SCHUETZIA BENNIEANA (KIDSTON).—

This Palaeozoic fructification which is described and figured by Kidston<sup>1</sup> on p. 424 and Pl. cvii, Figs. 7-13, was regarded by him as an aggregate of pollen sacs. The



FIGS. 1-3. 1 and 2 show a single tetrad as seen in two successive sections. The line on right is  $19\mu$  in length. 3 shows a tetrad complete and drawn to same scale as FIGS. 1 and 2.

present Note revealing more of their structure and the presence of tetrads of small spores  $20\mu$  across, sheathed in cuticular envelopes, is put forward as a contribution to the evidence against Schuetzia being a pollen-apparatus.

The discovery is due to Dr. Halle's new method of treating the incrustations of fossil plants of the Primary Rocks. Halle describes his new technique on pp. 5-7 of his recent Treatise.<sup>2</sup> It is sufficient for the present Note to state that he has found a means of softening the carbonaceous matter sufficiently to enable him to embed the material and section it with a microtome. As he justifiably says:—'the sections will be found in several cases to provide information on the arrangement of the sporangia which in material of this kind could be obtained by no other method'.

Kidston has figured the 'spores' he found in Schuetzia under two different magnifications—in his article those in Fig. 11 are magnified seven times and those in Fig. 13 are  $\times 200$ . This has, I believe, given rise to the statement that he found 'small spores'. All the spores described by Kidston are large, for they are from  $50-70\mu$  across.

<sup>1</sup> Kidston, R.: Memoirs of Geol. Survey, Great Britain, ii, 1924.

<sup>2</sup> Halle, T. G.: The Structure of Certain Fossil Spore-bearing Organs believed to belong to Pteridosperms. Kungl. Svenska Vetenskapsakademiens Handlingar Tredje Serien. Band 12, No. 6.

We are then confronted with the problem of two types of spore occurring in one fructification, and that fructification hitherto always interpreted as a pollen synangium. As this is no longer a possible interpretation of *Schuetzia*, it is suggested that the fructification is of ovular nature and that the tetrads of small spores ( $20\mu$ ) gave rise on germination in slightly older stages to embryo-sacs (Kidston's 'spores'). This interpretation is consistent also with several new specimens of these fructifications which are found bearing seeds in considerable number. A description of the latter is shortly to be published.

M. BENSON.

**THREE METHODS OF USING COTTON BLUE AS A MYCOLOGICAL STAIN.**—Cotton blue has frequently been advocated as a stain for fungal hyphae. The stain may be incorporated either in glycerine or lactic acid, and the material to be examined mounted in the coloured liquid or, after staining, transferred to glycerine jelly. When a solution of cotton blue in lactic acid is used as a mounting medium, general morphological structures are very clearly differentiated. In the following article three methods are described which may be employed in preparing permanent preparations of mycelium *in situ* or in the direct examination of fungal hyphae in woody tissues by means of vertical illumination.

(1) Cotton blue is a favourable general cytoplasmic stain for all Phycomycetes, and may well be employed to study the early stages in the formation of the sexual organs of oomycetes growing in culture. The following method has given particularly successful results.

A clean microscope slide is sterilized in a Petri dish which is then filled with about 30 c.c. of clear agar medium. This produces a thin layer over the surface of the slide which should be arranged so that the centre of the slide coincides with the centre of the Petri dish. The medium is now inoculated and incubated. After a suitable period the mycelium in the agar may be stained after fixation with 1 per cent. chromo-acetic acid. The best results have been obtained by placing a Petri dish containing the fixative in the incubator, and when the fluid is warm transferring the thin layer of agar with its supporting slide to the fixative. The slide and agar may be easily dislodged by cutting round the edge of the slide with a scalpel and prising it up from the bottom of the dish. Allow the mycelium to fix for 24 hours at incubator temperature, and then wash thoroughly in gently flowing water. When free of fixative the slide and agar may be placed in 10 per cent. glycerine which is then allowed to concentrate to the strength of Amann's medium. This latter is prepared by dissolving in order,

Carbolic acid crystals	20 grm.
Lactic acid syrup	20 „
Distilled water	20 „
Glycerine (pure)	40 „

Transfer the agar film to a small quantity of this medium in which 0.5 per cent. of cotton blue has been dissolved. Stain for from 6 to 24 hours, according to the

nature of the fungus employed, and then differentiate in several changes of Amann's medium till no further colour is removed from the hyphae. Transfer to 70 per cent. alcohol to wash out the Amann's medium, and bring up through 95 per cent. alcohol to dehydrate in absolute alcohol. If these stages are carefully carried out the agar will still adhere to the slide. Transfer from absolute to  $1/3$  xylol in  $2/3$  alcohol, to  $2/3$  xylol in  $1/3$  alcohol, and finally clear in pure xylol. When clear, infiltrate the preparation with balsam dissolved in xylol, and allow the excess balsam to drip off the slide. Trim up the edges of the preparation and cover with a large (2 in.  $\times$  7/8 in.) cover-glass.

Preparations made in this way give very good results, and if fixation and differentiation have been thorough, nuclei may be defined by their more intense blue colour. The stain is permanent for at least four years, even if the slides are regularly exposed to light.

The method may be used to investigate early stages in the formation of certain perithecia and pycnidia, but in most cases of this kind the erythrosin staining method described by Gwynne-Vaughan and Barnes has given better results.

(2) The differentiation of fungal hyphae penetrating through woody tissues can be secured by staining them with cotton blue and counterstaining the xylem with safranin. The method described here works equally well with fresh or fixed hyphae and tissue, and may be used to advantage in demonstrating the presence of fungal mycelium in structural timber.

Thin sections of infected tissue are placed in a slightly warm 0.5 per cent. solution of cotton blue in Amann's medium for 5 to 15 minutes. The excess stain is removed by washing in one or two portions of Amann's medium, which is then removed by washing in 70 per cent. alcohol. Transfer the sections from this to a solution of safranin (equal parts of a saturated solution of water-soluble safranin and of a saturated solution of alcohol-soluble safranin). Allow to stain for about 10 minutes. Wash out most of the excess stain in 70 per cent. alcohol and transfer the sections through 95 per cent. alcohol to absolute alcohol. After dehydration clear in xylol and mount in balsam.

The protoplasm of the hyphae stains a brilliant blue colour, contrasting with the red of the xylem. Differentiation is sufficiently marked to yield preparations which can be photographed, and in many cases penetration of the walls of tracheae can easily be observed.

(3) The examination of superficial mycelium by means of low-power objectives using vertical or direct illumination has regularly been employed in mycology, but a similar method of investigating the presence of hyphae upon cut surfaces of infected tissue, and especially timber, has not become a routine practice. This is partly because the normal illumination employed limits the power of objective which can be utilized, and partly because the hyphae are not easily differentiated from the host tissues. By using the Ultropak<sup>1</sup> vertical illuminator, after a pretreatment of the cut surface of the infected tissue with cotton blue, fungal hyphae can be observed under the highest dry objectives, and even under oil immersion objectives.

The method described below has been employed chiefly with woody stems and

<sup>1</sup> Made by Messrs. E. Leitz.

structural timber, storage organs and fleshy fruits, but it may well be extended to herbaceous stems. The infected block of tissue is split through the middle in a radial direction and the cut surface smoothed off with a sharp razor. The prepared surface is then immersed in slightly warm cotton-blue solution in Amann's medium (a 0.5 to 1.0 per cent. solution) for about 10 minutes, and the surplus stain is then washed away in Amann's medium. The block is now placed on a microscope slide and the prepared surface covered with a cover-glass. In the case of woody tissue the infecting hyphae are differentiated by their blue colour against the white of the background, while in storage organs the hyphae are more intensely blue than the general ground tissue.

Under a low power this method can be employed in a qualitative manner to indicate the degree of penetration of fungi inoculated on the surface of wood blocks, storage organs, or fruits. It has been employed to demonstrate the effectiveness of commercial timber preservatives in preventing the attack of wood-destroying fungi. The great asset of the method is the speed with which numerous routine examinations can be carried out.

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# A Preliminary Contribution to the Structure and Development of *Coenogonium Linkii*.

BY

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With Plates XVI to XVIII and nineteen Figures in the Text.

TO students of the Thallophytes one of the most striking appearances of plant growth in tropical regions is the green bract lichen *Coenogonium*. Its presence around small saplings, twigs, vines, and on the bark of large trees, together with the concentric zonation and habit of growth, remind one at once, except for the brilliant chlorophyll green colour, of the bracket fungi. This lichen has been observed in great abundance near the ground in all parts of British Honduras from the Orange Walk to the Toledo district, and in southern Mexico and the Peten district of Guatemala; and because of its striking appearance the writer was led to a study of the constitution, morphology, and general life-history of this organism. It occurs abundantly and is particularly conspicuous during the rainy season, but in the dry period from January to June it becomes less evident as a result of the extreme desiccation to which it is subjected. *Coenogonium Linkii*, as described in the literature, occurs generally throughout the tropics on mosses, vines, twigs, and the bark of trees in damp places, and in view of its wide distribution and characteristic appearance it is remarkable that this lichen has not received more intensive study. The majority of studies up to the present time have been primarily systematic and relate largely to dried herbarium material.

The present study was made during the period 1927-32, spent by the writer in tropical America directing the Chicle Research Project of the Tropical Plant Research Foundation. Except for the sections of apothecia and pycnidia, the data here given relate entirely to living material. *C. Linkii* appears to withstand considerable desiccation without injury, and for this reason it has been relatively easy to transport in the living condition to the States for intensive study. The algal constituent grows fairly well on Detmer's agar and can be transferred directly to this medium. Foreign

algae and fungi which might be introduced in transfer grow very slowly, and have not been a serious handicap. In this condition the alga has been kept growing in the laboratory for more than a year. Seedlings of various kinds with the lichen attached were also imported and planted in the tropical greenhouse at Columbia University, and under such humid and warm conditions *C. Linkii* has grown remarkably well.

#### LITERATURE REVIEW.

The literature dealing with *Coenogonium*, and particularly with its algal constituent, is extensive, and has been amply brought together by Zahlbruckner (97). A detailed review would thus doubtless be superfluous, but in view of the fact that this lichen is very similar in superficial appearance to, and has been confused with, *Cora pavonia* and *Dictyonema sericeum*, tropical Hymenolichens, it seems, none the less, worth while to review the essential data on structure and development. The family Coenogoniaceae is generally included with the Ascolichens, and according to Hue (38), Engler and Prantl (97), Smith (80, 81), Wainio (86), Zahlbruckner (98), Engler (99), and others, consists of approximately thirty species, varieties, and forms widely distributed throughout the tropics and occurring sporadically in the warmer and humid regions of the temperate zones.

The genus *Coenogonium* was established by Ehrenberg in 1820 on material collected in Brazil with *C. Linkii* as the type species. Ehrenberg gave small but excellent figures of the thallus, apothecia, asci, ascospores, and filaments of the algal constituent. Additional studies by other lichenologists soon substantiated his classification of this plant as a lichen. In 1851 Montagne (54) described a variety of *C. Linkii*, var. *Leprieurii* from material collected by Leprieur in Guiana during the period 1835 to 1849. He placed *Coenogonium* in the family Collemaceae which was made synonymous with Fries' Byssaceae. Later (1857) he added another species, *C. Tuckermanni*, from Venezuela. In 1857 K. Müller added a new species, *C. Echinus* from Brazil, and two years later Nylander described two additional species, *C. complexum* and *C. confervoides*, from Bolivia, Tahiti, and other islands of the South Seas, and included *Coenogonium* in the tribe Lecidinei. In 1860 H. Karsten described another species, *C. andinum*, collected in Venezuela, and gave excellent figures of the structure of the thallus and apothecia. His description of the development of the discocarps, however, was so fanciful and hypothetical that he was at once discredited. Karsten compared the young apothecium to an archegonium and described fecundation as taking place in the same manner as in the cryptogams in general. It soon became obvious from subsequent studies that what he mistook for the rudimentary apothecium is merely a young globular branch of the filamentous gonidium, *Trentepohlia*, and that the

cortical envelope of surrounding filaments which he believed contained spermatia and functioned perhaps as antheridia is but mycelium. Karsten's contentions were shortly attacked and completely discredited by Schwendener (76, 77) and Nylander (66). Schwendener (76) described and figured several stages in the development of the young apothecium to complete maturity and showed its relationship to the gonidia. His figures are rather small, but they show, none the less, that the branches of the gonidia are not concerned in the formation of the apothecia, and may merely become enveloped in the process. Nylander (67), in 1862, in addition to pointing out the misrepresentations of Karsten, monographed the genus describing ten species, five of which were new. Bornet (4) gave an excellent description of the gonidia of lichens in 1873 and illustrated their relation to the fungus constituents in detail with beautiful coloured plates. Among his descriptions and figures is *C. confervoides*, which is shown to be composed of filaments of *Trentepohlia* enclosed in a sheath of closely applied, branched and anastomosing fungus filaments.

The remaining studies on *Coenogonium*, with the exception of those of Hariot (32), Reinke (73), Glück (28), Simmer (79), and Smith (80), have for the most part been primarily systematic, and little further fundamental data on structure and development have been added. Thwaites (82), K. Müller (64), Tuckerman (83, 84, 85), Hooker (37), Leighton (48), Willey (93), Nylander (68), Krempelhuber (43, 44, 45), Bailey (1), Möbius (53), J. Müller (56, 57, 58, 59, 60, 61, 62, 63, 64), Wainio (86, 87, 88), Hue (38), Hellbom (35), Smith (80, 81), Lindau (49), Zahlbruckner (95, 96, 97, 98, 99, 100, 101), and others have added new species, or listed and redescribed old ones from various tropical and temperate zones throughout the world.

The thallus of *C. pavonia* and *D. sericeum*, Hymenolichens which also occur around twigs, branches, vines, saplings, &c., in tropical regions, is closely similar in external appearance to that of *Coenogonium*. It is usually round in contour, somewhat saucer-shaped and felt-like, concentric in growth and blue-green in colour, and is also closely similar in general appearance to the bracket fungi. In fact, Woodward (94) expressed the belief that *C. pavonia* belonged to a new genus of fungi close to *Polyporus versicolor*, and Johow's figures (39) likewise strongly emphasize this bracket-like appearance. For these reasons *Cora* and *Coenogonium* were doubtless confused by Nylander, a fact which led to considerable subsequent controversy. Fries (25) established the genus *Cora* in 1825 to include forms which had been classed with the algae in the genus *Ulva*, and placed it in the family Byssaceae near the genera *Lichina*, *Cilicia*, *Coenogonium*, &c. Nylander (66) was one of the first to observe the presence of gonidia in this plant and demonstrated its true lichen structure. He also gave a detailed description of apothecia, asci, spores, and paraphyses, and thus shifted *Cora* from the Hymenomycetes to the Ascolichens. In 1855 he

placed the genus among the Lecanorei; later he shifted it to the Psoromei, and finally fixed its position among the Pyrenocarpei. In this manner the controversy concerning the two genera began. There is little doubt, as has been shown from subsequent investigations, that Nylander was in reality studying thalli of *Coenogonium* when he described apothecia for *Cora*. The material which he studied was a collection from Bolivia, and it is highly probable that thalli of *Coenogonium* were accidentally included among those of *Cora*.

Mattirolo (50) made an extensive study of *C. pavonia* to check the observations of Nylander and failed to discover apothecia in any of his material. He shifted the genus to the Hymenolichens, and regarded the fungus constituent as belonging to the Auriculariaceae near *Thelephora* and *Hypochmus*. Mattirolo's observations were confirmed in 1884 by Johow, who showed conclusively that *Cora* and *Coenogonium* are distinct genera, and belong to widely different groups of lichens.

#### GENERAL MORPHOLOGY OF THE LICHEN THALLUS.

The thallus of *Coenogonium Linkii*, as has been described by previous investigators, consists of numerous branched, more or less parallel filaments which are united to form a flat, somewhat saucer-shaped, bracket-like felt tissue, as is shown in Pl. XVI, Fig. 1. Its colour, brilliant chlorophyll green, makes it conspicuous in the jungle. It may encircle the twig, vine, or branch on which it is growing if these are small. The sapling whose portion is shown in Pl. XVI, Fig. 1, had as many as twelve bracts to a height of 18 in. The thallus varies considerably in size according to age, and shows definite and beautiful zonations or rings of growth. Whether or not these zones represent annual growth periods is uncertain in the Central American material, but they have been so regarded by the students of *Coenogonium*, *Cora*, and *Dictyonema*.

The zones in my material are marked by differences in thickness of the thallus, which are perhaps due to periodic variations in growth. The denser zones are considerably thicker than the remainder of the thallus, and appear somewhat elevated like concentric ridges. These elevations become greater from the margin towards the centre, and in older and larger specimens it may be observed that new overlapping felts or mats originate in these zones or ridges. These continue to grow, and as a consequence the large and mature thalli are frequently made up of a number of overlapping felts which give them the inverted cup- or saucer-like appearance shown in Pl. XVI, Fig. 1. The view that the denser zones are associated with periodic variations in growth is supported by the profuse branching of the algal filaments in those regions. However, between the conspicuous zones occur others thinner, narrower, and less definite, and it



is therefore problematical whether all of these more or less concentric rings represent specific annual growth periods. The transition between the wet and dry seasons in British Honduras is none the less very marked, and relatively dry periods may occur during the rainy season; so that as far as rainfall is concerned the conditions are optimum for marked variations in growth.

The thalli vary considerably in size according to their age and location. Specimens with mature apothecia have been found to vary from 3 to 25 mm. in diameter. It is thus difficult to use this character alone as a basis of classification. The apothecia appear in great abundance on the under side and are waxy, yellow to light orange in colour when fresh. On pressed and dried material, however, they may turn to light brown within a year's time. At what age of the thallus the apothecia first appear is uncertain from my observations. The thalli grow and persist for several years, and whether or not the fungus fruiting bodies appear annually I am unable to say at present.

The filaments which compose the thallus consist of two kinds of cells, a central row of large cylindrical *Trentepohlia* cells and numerous peripheral, hyaline, septate, mycelial strands which branch and anastomose to form a net-like structure around the central row. If these filaments are examined under the high powers of the microscope, the relation and type of branching of the two constituents is readily visible. Pl. XVI, Fig. 10, shows a portion of a filament of *Trentepohlia* with the surrounding mycelial threads. It is to be noted in this figure that the fungus constituent is very closely applied to the cells of the alga; and in surface views such as are shown in Pl. XVI, Figs. 11 and 12, it makes a complete network around the algal filament. Frequent anastomoses between the mycelial filaments as well as numerous branches make up this net-like structure. As was noted and figured by Bornet, the fungus filaments may be denser and more reticulated near the base of the thallus, and almost completely obscure the algal cells. Text-fig. 12 shows an enlarged portion of the thallus near the region of attachment in which the mycelial filaments anastomose, branch, and run from one algal filament to another. Ordinarily the ends of the *Trentepohlia* branches are free, particularly during a period of rapid growth, since the fungus filaments then appear to lag behind (Pl. XVI, Fig. 19 a). If the algal growth is very slow, however, the hyphae may extend up to and around the tip, as shown in Pl. XVI, Figs. 10 and 12. The number of hyphae around each algal filament varies. Pl. XVI, Fig. 19, shows a cross section from fixed and stained material with five strands, two of which have anastomosed. The fungus is particularly abundant around the young branches, and may often be several cells in thickness as is shown in Pl. XVI, Figs. 10 and 18, and has also been figured by Johow. The pressure of the growing branch appears to stimulate the fungus to greater growth and

division in these regions, and we have here perhaps another case of distinct functional hypertrophy. That these snarls of hyphae are not always rudiments of apothecia, as Karsten and Schwendener figured, is evident when a large amount of material is examined. Apothecia may or may not develop at such places, while the majority of young branches are almost completely enveloped, as is shown in Pl. XVI, Fig. 10.

In none of the thalli and filaments so far observed have haustoria been found. The fungus grows very closely applied to the gonidia as noted above without becoming at all intracellular, and no injurious effects on the algal cells have yet been seen. The fungus, none the less, appears to have some inhibitory effect on the branching habit of the alga. However, the shape and characteristic development of the lichen thallus seems to be determined primarily by the growth habit of the alga. It is the alga which makes up the bulk of the thallus and possibly dominates development. Aside from the doubtfully inhibitory effect noted above and the wafting or binding together of the algal filaments (Text-fig. 12) near the base, the fungus mycelium appears to parallel the growth of the alga. It must, nevertheless, be borne in mind in this connexion that the alga when grown apart from the fungus, never, as far as my observations go, produces a concentric thallus exactly like *Coenogonium*.

## MORPHOLOGY AND LIFE-HISTORY OF THE ALGA.

### *Vegetative Structure and Modifications.*

*Trentepohlia* sp., which is the gonidium of this lichen, is made up of richly branched, septate filaments which grow principally by the division of the apical cell. The branching is very profuse in some thalli and less so under certain environmental conditions. In Pl. XVI, Fig. 10 is shown the habit of branching as well as the size and structure of the cells. This figure is characteristic of the alga in close association with the fungus near the border of the thallus, but, as will be shown later, the alga undergoes considerable change in habit of growth when cultured on synthetic media apart from the mycelial filaments.

The individual cells vary greatly in length and diameter, and it is very difficult to identify the species on this character alone. Numerous measurements of cells in different parts of the lichen thallus and under different cultural conditions have shown a wide range of variation. Some cells may be isodiametric near the base of the thallus, as is shown in Pl. XVI, Fig. 19 *a*, while at the periphery the length may be as much as six times the diameter, which is well illustrated in some of the end branches in Pl. XVI, Fig. 10. In my material the variations range from  $10 \times 10 \mu$  in the isodiametric to  $38 \times 6 \mu$  in the longest cells.

Each cell contains numerous brilliant green plastids which vary greatly in number, size, and shape. Ovoid, elongate, somewhat band-shaped, and irregular plastids may be found in the same filament and often in single cells. Pl. XVI, Fig. 13, shows some of the variations which occur in this species. Schmitz (75 a) figures the plastids of *Trentepohlia* as small flat panes or discs. Karsten describes them in general as round and oval discs in mature cells, but also figures wide differences in size and shape, and this observation has been confirmed by most subsequent workers. In young cells of *T. bisporangiata* the plastids are figured as elongate, irregular bands which break up into smaller, more or less rounded bodies. Heering (34), Printz (71), Oltmanns (69 a), and others likewise describe similar variations and thus confirm Karsten's observations. Geitler (27) describes two general types of plastids in *Trentepohlia*, plate- or disc-, and band-shaped, which may change from one to the other under certain conditions. Species with cylindrical cells possess chiefly plate-shaped plastids, while those with round or oval cells have band-shaped chromatophores.

I have also, as noted above, observed somewhat similar variations, but have so far been unable to correlate them definitely with age and development. However, in view of the fact that plastids are but regions of the cytoplasm impregnated with chlorophyll which may grow in size with maturity and divide and thus become specialized for specific function, such changes in size and shape of these organs are highly conceivable. As many as forty distinct plastids have been found lined up end to end in a single elongate cell. Again, they may be found running in rows diagonally across the cell, and under such conditions, and particularly when they are numerous and crowded, one may be led to interpret the plastid as a somewhat reticulate structure. That this is not so is evident from observations of long cells with few plastids. Here they may be oval, round, quite distinct and separate. The round and oval shapes are not necessarily correlated with age as far as my observations go. In all of the germinating zoospores which have been found the plastids were not elongate and band-shaped, but rather small, oval, and round. No pyrenoids or starch grains have been observed in association with the plastids.

In addition to the plastids numerous hyaline, yellow and golden-red, highly refractive globules or bodies of various sizes occur in the more viscid cytoplasm and vacuoles. These bodies have been interpreted in other species as haematochrome, oil, and various lipid compounds by Hildebrand (36), Cohn (10), Bornet (4), Wille (91), Hariot (31, 32, 33), Karsten (41), and other students of this group. In more recent years Oltmanns (69 a), Fritsch (26), Meyer (51), Heering (34), Fischer (24), van Oye (70), and Biswas (2) have regarded them as light filters which increase with intense illumination and decrease in size and number when the cells are shaded. Fritsch and van Oye in particular regard haematochrome

formation as one of the most essential factors which enables *Trentepohlia* to withstand the intense illumination of the tropics and thus compete successfully with other algae. Senn (78) and Geitler (27), on the other hand, believe that these globules are reserve food material in the same sense as starch and proteids, which are built up and used in the normal metabolism of the cell.

In my material as many as a hundred globules of various size have been counted in one cell. As is shown in Pl. XVI, Fig. 14, they vary in the same cell from tiny droplets to large spheres. The size, number, and coloration appear to be associated, as Meyer, Geitler, and others have shown, with the age of the cell and the condition under which the alga is growing. They seem to arise as very small droplets which may coalesce to form larger globules or may grow independently in size. They appear more hyaline and refractive when first formed and then gradually take on the yellow and golden-red colour. When very abundant they may somewhat mask the chloroplasts and impart a yellowish or golden tinge to the cells. In young cells at the tip of growing filaments, or in germinating zoospores and sporelings growing on Detmer's agar, such as are shown in Pl. XVI, Figs. 16, 17, Pl. XVII, Figs. 21, 25, 26, 53 and 54, they are usually less abundant and more hyaline in colour. Thalli collected in more exposed regions in the jungle were very rich in globules of this nature, but when they were transferred to moist chambers and agar cultures in the laboratory the number, size, and coloration decreased perceptibly. These observations confirm those of Meyer, Senn, Fischer, van Oye, and Geitler that haematochrome is less abundant in agar cultures and in decreased illumination.

The cell-wall of *Trentepohlia* sp. is fairly uniform in thickness and consists apparently of several layers (Correns (11)). No cellulose caps, such as have been described by Caspary (7), Karsten (42), Brand (5), West (90), West and Hood (89), and others at the apex of the filaments, have yet been observed in my material.

When bits of the *Coenogonium* thallus were transferred to Detmer's agar, the fungus constituent ceased to grow, died, and finally disappeared within eight weeks, leaving the alga growing independently. I have succeeded in growing this species of *Trentepohlia* on such media for more than a year and have observed considerable modification in its habit of growth under those conditions. In my material branching tends to become more free at once. As a general rule the cells become either shorter in length and greater in diameter, or elongate to a great extent and take on a more brilliant green colour. This latter change in appearance may be partly due to the absence of the fungus mycelium and to a difference in the yellow and golden-red globules noted above. These globules are usually less in number and more hyaline and colourless. The chloroplasts which

were almost completely obscured by the red granules thus become distinct and clear.

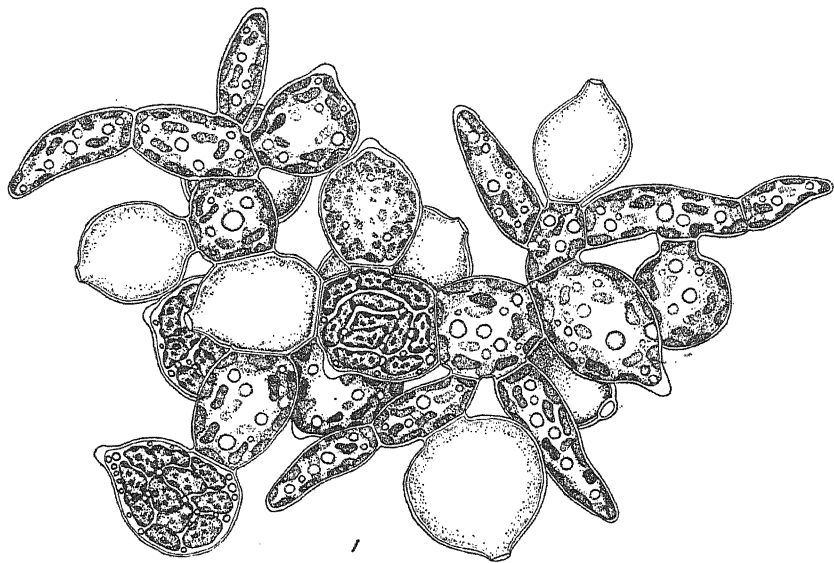
The algal growth shown in Pl. XVII, Fig. 20, is fairly characteristic of that developed under cultural conditions. It is to be noted that the central axis is less conspicuous than in Pl. XVI, Fig. 10, and that the branches go out in all directions. This greater tendency to branch in all directions appears to be associated to a certain extent with the absence of the fungus mycelia. As is shown in this figure and as has been previously described by Schwendener, Bornet, and Johow, the fungus develops strongly where new branches are beginning to form. Often it may be several layers in thickness in such regions, as is shown in Pl. XVI, Figs. 5, 10, and 18. It seems as if this abundant growth inhibits the development of branches to a certain degree and thus produces the characteristic appearance of the algal filament in the lichen thallus. When grown apart the inhibitory factor is absent, and this may account for the profuse branching and more radial habit of growth of individual algal thalli. However, that this factor is not the dominant one in determining the habit of growth of *Trentepohlia* in the lichen is clearly evident from observation of other species growing independently in nature. The non-lichenic species as described in the literature branch freely and tend to grow more or less radially.

A younger but similar filament is shown in Pl. XVII, Fig. 21. The cells are comparatively small and somewhat moniliform in shape and general appearance; and were such thalli found growing isolated and independently in nature, they might readily be mistaken for a different species. The shape of the cells shown in this figure is somewhat suggestive of *Physolinum monile* described by De Wildeman (16, 17, 18), Karsten (41), and Printz (71).

The most extreme variation so far observed under cultural conditions is shown in Text-fig. 1. Here almost the entire thallus consists of unusually large cells, the majority of which have developed into zoosporangia. No less than thirteen sporangia are present in this thallus, and some of the vegetative cells are fully four times the diameter of the smaller ones shown in Pl. XVI, Fig. 10. When this thallus was first discovered I thought at once it was *P. monile*, inasmuch as Printz (71) reports its association with other species of *Trentepohlia*. However, from subsequent study of its growth, manner of zoospore production, and size of the latter, I am inclined to regard it as nothing more than a modification of *Trentepohlia* sp. No aplanospores such as those described by Printz were observed, and the zoospores were formed in the same manner and were of the same size and general appearance as in the gonidium of *C. Linkii*. Furthermore, the end branches of the thallus are definitely cylindrical and not moniliform as in *P. monile*.

The increased tendency to sporangial development or reproduction when grown apart from the fungus has been evident in most of my

cultures, but I am not certain whether this is only a periodic occurrence or is due to the change in environment. Continued observations of this species throughout the seasons in nature have not been made, and it is impossible to say at present what its periodicity is. However, sporangia



TEXT-FIG. 1. Thallus of *Trentepohlia* sp. from Detmer's agar culture.

have been found in great abundance so far at all times during the past year in my agar cultures. The terminal portion of a branch with eight sporangia in various stages of development, as shown in Pl. XVI, Fig. 15, is fairly characteristic of such cultures.

The structural and morphological modifications under the cultural conditions noted above are doubtless to a certain degree abnormal and make identification of this particular species very difficult. Towards the end of a year the filaments growing on Detmer's agar were quite unlike those in the lichen thallus with respect to branching, size, and shape of the vegetative cells, and zoosporangia; and the two thalli could hardly be recognized as the same species. The Trentepohliaceae are largely terrestrial algae, and when grown under different conditions show a high degree of variation, as has been noted by all students of these algae. Gobi (29), Hansgirg (30), De Wildeman (15), Deckenbach (14), Wille (91), Schmidle (75), Brand (5, 6), Meyer (51), and others have described the extreme variability of these algae in cultures. Hansgirg and Deckenbach particularly have emphasized these changes and claimed that polymorphism is common among the Trentepohliaceae. Deckenbach questioned the validity of *T. umbrina* on this basis, and claimed that it is only a resting form of

*T. aurea*, which by further development goes over into a *T. lagenifera* and finally into a *T. uncinatus* form. He thus combines the four forms into a polymorphic species which is named *T. polymorpha*. *Trentepohlia* sp. also varies considerably in nature during the wet and dry seasons in Central America, and it is often impossible to determine which characters are specific. Particularly is this true when the *Coenogonium* thalli occur near the ground in damp places and are partly submerged in the rainy season. Under such conditions variations as extreme as those described from agar cultures frequently occur.

Möbius (53) reports that this species was described by Bornet as *T. flavum*, but in the reference which he gives it is figured as the algal constituent of *Chiodecton nigrocinctum* instead of *C. Linkii*. De Bary (12) described *C. nigrocinctum*, *Byssocaulon*, and *C. Linkii* as a single lichen with Kützing's (46), *Trentepohlia* (*Chroölepus*) *flavum* as the algal constituent, and this is doubtless where Möbius gets the specific name for his Porto Rican material. Schwendener (76) describes the cells as being 16 to 18  $\mu$  in diameter and three to four times as long. My measurements are considerably smaller and correspond more closely with those given by Möbius. They also fall within the limits for *T. dialepta* as given by Nylander (67), Hariot (31), and De Wildeman (22). However, in view of the structural modifications noted above, no attempt is here made to identify this species of *Trentepohlia*. Further studies on the morphology, development, and cytology are now in progress and will be reported later.

#### *Reproduction.*

In addition to zoospores and gametes, *Trentepohlia* sp. reproduces and regenerates the thallus by aplanospores, isolation of vegetative cells, and other similar means. Rather frequently after the lichen thalli had been transported to the States and transferred to Detmer's agar, entire algal filaments with the exception of a few terminal and intercalary cells died, and in such cases these living cells continued to grow and regenerated new and extensive thalli. A typical example is shown in Pl. XVII, Fig. 22, in which only the branches and apical cells are alive. This filament was grown in a hanging drop chamber on Detmer's agar for more than two months, and in the meantime the apical cells produced a well developed thallus. This regenerative ability of individual cells is undoubtedly an important factor in aiding the alga and lichen to recover after the dry season and long periods of desiccation.

In numerous thalli growing in nature and on agar cultures it has been found that individual or several intercalary cells may die and become empty, and in such cases the adjoining cells frequently pierce, grow into, and completely fill the empty cells, as is shown in Pl. XVII, Fig. 23. Such growth is similar to the phenomenon of 'Durchwachsung', which is wide-

spread among the fungi, and has also been described and figured by De Wildeman (15) and Brand (5) for *Trentepohlia*. Another example is illustrated in Text-fig. 2. Two of the cells in the centre of the filament have died; the adjacent cells on each side have grown into them, broken through the lateral walls, and developed upwards into short-branched filaments. Proliferation of empty zoosporangia has been observed in three filaments of *Trentepohlia* sp. in a manner similar to that described by Schmidle for *T. ellipsicarpa* and by Reinsch (74) for *Acroblaste*. In each case the stalk cell pierced the base of the sporangium, grew into it, and formed a secondary sporangium. In one instance the proliferating cell failed to form a zoosporangium but grew out into a long multicellular filament. Such growth is fundamentally similar to 'Durchwachsung', and doubtless belongs in the same category.

Individual cells and short fragments may break away from filaments and grow independently (Pl. XVII, Figs. 25, 26, 27, and 28). This occurs frequently in nature also, and is hardly to be regarded as a distinctly cultural characteristic. Pl. XVII, Figs. 26 and 27, show the development of such a cell over a period of three weeks. Division occurred the first week, and one of the cells developed into a zoosporangium. Pl. XVII, Fig. 28, shows a small thallus with two sporangia which developed from a short fragment. The walls of these cells are of the same thickness as those of normal filaments, and thus appear different in this respect from the akinetes figured by Meyer for *T. umbrina*. Numerous round sporangia have also been found isolated from filaments in agar cultures as well as in nature. If lichen thalli are transferred to damp filter paper, and kept thus in a moist chamber, large numbers of isolated sporangia and vegetative cells may subsequently be found adhering to the paper. Such sporangia have been kept under observation for several weeks, and found to undergo cleavage into zoospores. This appears to occur commonly in nature also. During the dry season such sporangia become invested with rather thick walls, and may perhaps be regarded as resting sporangia. They appear able to withstand considerable desiccation, and doubtless serve to disseminate the alga. When such sporangia are placed in water they germinate readily by the production of zoospores. Whether or not they are detached from the thallus by ring-like thickening in the manner described by Brand (6) I am unable to say.

Frequently in nature the contents of intercalary and other cells may round up, and become invested with a new thick wall, as is shown in Pl. XVII, Fig. 24. The cell here shown is very large in size, and has burst the old wall of the filament. Such cells are like the aplanospores of other algae, and doubtless serve the same purpose in this species of *Trentepohlia*. They have frequently been mounted and studied for long intervals of time, but so far I have been unable to observe germinations.



*Structure and Development of the Zoosporangia.*

The zoosporangia may be terminal, lateral, or intercalary, with or without stalk cells, solitary or in clusters on short branches. In nature they are not formed abundantly as far as my observations of the Central American material go, but as soon as the filaments are transferred to water or agar cultures, sporangia are produced in great abundance. Pl. XVI, Fig. 15, shows the characteristic development in agar cultures within a few weeks after transfer. Sporangia may sometimes be borne so profusely that they appear to be borne in clusters at the end of a filament. Pl. XVII, Figs. 31-40, show terminal, lateral, stalked, sessile, and isolated sporangia of various sizes and shapes. The majority are oval, round, somewhat egg-shaped, and slightly beaked, but as the agar cultures begin to degenerate, considerable variations occur. In nature the zoosporangia vary from  $14\ \mu$  in the spherical to  $18 \times 22\ \mu$  in the oval forms. If development occurs slowly the zoosporangia also may be partly enveloped by the mycelium, as shown in Pl. XVII, Figs. 31 and 32.

So far only globular sporangia (Kugelsporangien) have been observed. None of the hooked type (Hackensporangien) described by Gobi, Karsten, and Meyer, or the so-called 'trichtersporangien' figured by Brand (5, 6) have been found, either in nature or in culture. Most of my observations on sporangial development have been made on material gathered during the rainy season or grown on agar cultures, and this may perhaps account for the lack of hooked sporangia. Karsten (41) reported that such sporangia were soon transformed into globular forms when thalli were grown in water, and that the globular forms occurred most abundantly in moist places.

Considerable variation in the size and shape of the sporangia has been found under conditions of high moisture, and in agar cultures, particularly when the cultures become old. Under such conditions the sporangia have a tendency to elongate and become more flask-shaped, as is shown in Text-figs. 3 and 4 and Pl. XVII, Figs. 29 and 30. They are similar in shape, and fall readily within the dimensions of the sporangia of *T. lagenifera*. The zoosporangium shown in Pl. XVII, Fig. 30, occurred terminally, and was scarcely greater in diameter than the vegetative cells of the filament. Ordinarily a single papilla and opening is developed for the escape of the zoospores, but under the conditions noted above two and three may be formed (Text-figs. 5 and 6). In these sporangia the papillae are greatly elongated and similar to the sporangial necks of many chytrids.

The successive developmental stages of a sessile globular sporangium are shown in Text-figs. 14 to 19. The growth of a large number of similar sporangia has been followed in hanging-drop chambers, and development under such conditions is fairly uniform. It consists essentially of the

formation of a lateral bud or small branch which by subsequent growth is cut off by a wall, and enlarges into a more or less globular sporangium. Text-fig. 14 shows a very early stage in which the bud has just begun to

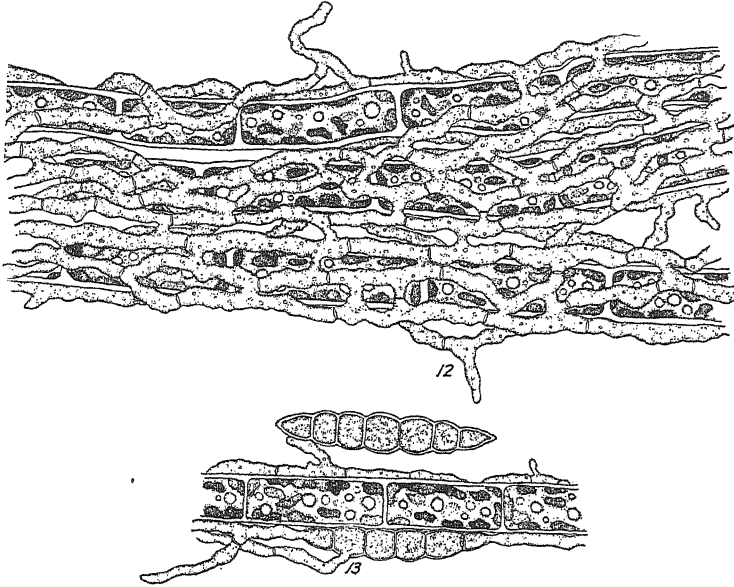


TEXT-FIGS. 2-11. Fig. 2. 'Durchwachsung' of dead intercalary cells. Figs. 3, 4, 5, and 6. Variations in the size, shape, and number of necks of sporangia from Detmer's agar cultures. Fig. 7. Large zoosporangium with non-motile spore segments escaping. Fig. 8. Pair of zoospores with cilia entangled. Fig. 9. Group of four zoospores stuck together. Figs. 10 and 11. Large 4-ciliated motile spore, or perhaps zygote, before and after coming to rest.

push out from the upper end of a vegetative cell. Its formation is in the early stages in no way different, as far as my observations go, from the development of a branch, and the two can be differentiated only by study of the successive developmental stages. In Text-fig. 15 is shown the same bud fourteen hours later. It has become slightly larger and separated by a transverse wall from the mother-cell. Text-figs. 16, 17, and 18 show subsequent stages, three, five, and eight days later. The changes consist chiefly of an increase in the number of plastids and globular bodies and enlargement of the developing sporangium itself. Increase in diameter continued until the twelfth day. On the tenth day the sporangial papilla appeared. Text-fig. 19 shows the final stage in development which occurred on the fourteenth day. Subsequent stages could not be followed for this sporangium, since the hanging-drop chamber dried out before cleavage and zoospore formation occurred. In many such cultures protozoa became fairly abundant, and a large number of mature sporangia disin-

tegrated, as if they had been injured by these organisms. The green protoplasmic contents would ooze out slowly into the water and soon become completely diffused.

Mature sporangia vary greatly in size and shape, and these characters



TEXT-FIGS. 12 and 13. Fig. 12. Portion of lichen thallus near region of attachment. The unguis mycelium makes a network around the algal filaments. Fig. 13. Large phragmosporous spores attached to *Trentepohlia* sp.

alone are not always indicative of the stage of maturity. Prior to cleavage their contents appear closely similar to those of ordinary vegetative cells. A large number of plastids of various sizes are distributed in the cytoplasm, as is shown in Pl. XVII, Fig. 31, together with numerous globules that are hyaline to orange-yellow in colour. Frequently sporangia in this stage may be found with a large central vacuole (Pl. XVII, Fig. 32) and a comparatively thin primordial utricle. In the transition from this stage to cleavage a considerable change occurs, particularly in the plastids. These structures begin to lose their definite delimitation (Pl. XVII, Fig. 33), so that the cytoplasm appears more homogeneous in its green granular structure. The boundaries of the separate plastids can be seen only by most careful observation. The chlorophyll appears to be more diffuse, but none the less is confined without sharp boundaries to the regions formerly occupied by the plastids. At this time there may be a slight contraction of the protoplast, so that the more definite plastids appear somewhat clumped at the periphery. Contraction appears to be due to loss of water, since a hyaline liquid is usually visible outside the denser protoplast. In

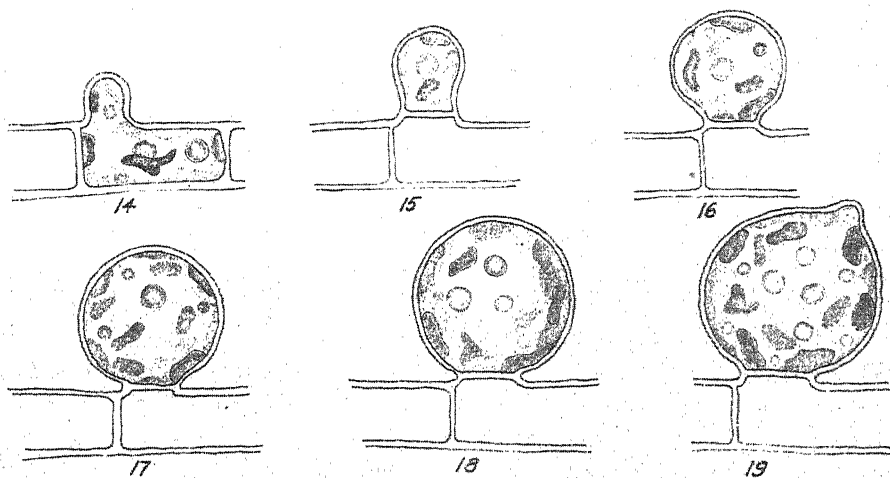
this process many of the refractive and coloured globules may be extruded. At least at the time of cleavage they are quite numerous at the periphery, and are usually lacking in the delimited spore segments. The loss of water and extrusion of these globules is doubtless a dedifferentiation of the protoplasm prior to reproduction, as has been described so often for other algae, fungi, and protozoa.

In Pl. XVII, Fig. 33, is shown an early stage in cleavage. The protoplast is slightly contracted and uneven at the periphery. The depressions on the membrane are doubtless the beginning of cleavage furrows. The plastids are not very definite in outline, and the cytoplasm has a more uniform but slightly lighter green appearance. A later stage is shown in Pl. XVII, Fig. 34. Here cleavage furrows are quite distinct, and are developing progressively from the periphery inwards. Doubtless, in the centrally vacuolated sporangia they also progress centrifugally, as has been so well shown by Bold (3) in *Protosiphon*. The plastids in this sporangium are less definite, and are represented by green granular areas. A few refractive globules are also still present in the protoplast. I have followed the progressive stages of cleavage beyond this phase in several sporangia, and am convinced that the process is not simultaneous as described by Meyer (51) in *T. lagenifera*. The furrows gradually cleave the protoplast into segments, and in no case have they been found to be delimited all at once. Pl. XVII, Fig. 35, shows a small beaked sporangium in which cleavage has been completed and the definitive spore segments are present. This is an optical view, and no attempt has been made to draw the segments lying below. At this stage, because of mutual contact and pressure the segments are usually pentagonal and hexagonal in shape. The plastids appear merely as small green granular areas without marked boundaries, and one is often led to question their presence as definite bodies. The globules which are not extruded prior to cleavage, and remain in the protoplast are rarely, as far as my observations go, included in the cleavage segments, but come eventually to lie at the periphery of the sporangium or between the incipient spores. None of the segments shown in Pl. XVII, Fig. 35, contain such globules. In *T. umbrina*, however, according to Gobi, Caspary, Brand, Meyer, &c., the zoospores contain numerous globules, and are usually brilliantly coloured.

Within a comparatively short time, depending on environmental conditions, the angular cleavage segments become more rounded and elongated, as is shown in Pl. XVII, Fig. 36. This is the normal shape of the zoospores just before they escape from the zoosporangium. The plastids in the meantime appear to have undergone further change, and are still less definite. The elongated cleavage segments lie in a hyaline liquid in which are also suspended the minute globules.

*Structure, Behaviour, and Germination of Zoospores.*

A few minutes after sporangia from agar cultures in which cleavage has been completed are placed in water, movement of the zoospore seg-



TEXT-FIGS. 14-19. Successive stages in the development of a sessile, lateral, globular zoosporangium.

ments begins. Almost simultaneously with this slight motion the papilla or short neck bursts, and the zoospores are ready to escape. The final bursting of the papilla appears to be due primarily to hydrostatic pressure from within. I have attempted on numerous occasions to study the details of this process, but because of the hyaline character of the sporangial wall very little in detail has been observed. At times it seems as if a dehiscence ring is formed whereby the cap is bodily lifted off as shown in Pl. XVII, Figs. 42 and 43, while in other sporangia there appears to be a definite tear (Pl. XVII, Fig. 41). In most of the empty sporangia, however, the edge of the mouth is quite smooth (Pl. XVII, Figs. 27, 28, 29, 37, and 55, and Text-figs. 1 and 6).

The swarm-spores first begin to glide slightly on each other, but this initial movement does not appear to be due to ciliary action, since the spores are usually so packed together as to prevent motion of these structures. However, the cilia are generally invisible at this stage because they are hyaline and the spores green; if cilia are present it is impossible to determine their movement. As the zoospores move about, some of the fluid within the zoosporangium exudes in a somewhat viscid mass, and often protrudes in advance, as illustrated in Text-fig. 3. This material, according to my observations, does not form a definite vesicle into which

the zoospores pass, as has been figured by Caspary (7, Figs. 6 and 8), but diffuses readily in the surrounding water (Text-fig. 7). If the mouth of the sporangium is very narrow the zoospores glide through slowly, and may stretch out to considerable length in passing through. Zoospores are often caught in the narrow orifice or become entangled with their cilia; in such cases they strain from side to side and undergo many changes in shape, as if making strenuous efforts to free themselves (Pl. XVII, Fig. 20 *a*). They may thrust out short pseudopods in various directions, then become much elongated, then contract. Frequently numerous contractile vacuoles appear under such conditions. On the other hand, if there are no mechanical obstructions the exit of the zoospores is easier and more rapid. I have not, however, found them escaping so rapidly but that they could be readily counted. The swarm-spores after their escape may remain fairly still for a second or two, and then dart off with lightning rapidity in various directions.

The zoospores follow one another through the orifice, as shown in Pl. XVII, Fig. 20 *a*, until the sporangium is empty. Sporangia are frequently found filled with non-motile resting zoospores. This condition seems to be due to the fact that the sporangia have failed to open, or the mouth has become obstructed in some manner. Frequently in my material zoospores came to rest without leaving the sporangium, although no mechanical difficulties or obstacles were visible. One sporangium of unusual size containing 170 zoospores was found and carefully observed in this respect. Sixty-seven zoospores escaped in regular fashion one after the other with great rapidity, and swam off immediately after becoming free. The majority of the remaining zoospores, however, gradually oozed out one by one in a mass of somewhat viscid substance, and immediately came to rest around the mouth of the sporangium without showing a motile stage. A large number remained within the sporangium and never escaped, although ample opportunity was present, as is shown in Text-fig. 7. These swarm-spores are considerably larger in size than the motile ones; this is characteristic, as will be shown later, of those which have come to rest. The sporangium shown in this figure was kept under further observation for more than an hour to watch the subsequent behaviour of these spores. Within ten minutes all of them had begun to disintegrate, and none formed definite walls around them and germinated later. The behaviour of the zoospores in this sporangium doubtless accounts for the common occurrence of large numbers of spores around the mouth of sporangia, a condition so frequently observed in cultures mounted in water. Whether this condition is associated with a lack of cilia or with immaturity I am as yet unable to say. None of the resting spores shown in Text-fig. 7 possessed cilia as far as could be determined in this hyaline material.

Frequently also numerous sporangia are found full of zoospores which are distinctly oval in shape and characteristically different in appearance from those which become motile. When sporangia with spores of the type shown in Pl. XVII, Figs. 38 and 40, were first found on the same filament with the form illustrated in Pl. XVII, Fig. 36, I thought at once that this condition indicated morphologically distinct gametangia bearing large and small swarm-spores respectively. Subsequent study, however, showed this view to be incorrect. The large oval zoospores shown in Pl. XVII, Figs. 28, 38 and 40, and Text-fig. 5 are similar to the others, it seems to me, except that they have failed to escape, and in coming to rest have become more spherical in shape and somewhat larger in size. The sporangia shown in Pl. XVII, Figs. 28, 38 and 40, have failed to open entirely, and the zoospores apparently had no opportunity whatever to escape. The contrast between sporangia of this type and the one shown in Pl. XVII, Fig. 36, is striking, and unless careful study were made of subsequent development one might be readily led to the conclusion that here were zoospores of two types. Meyer does figure and describe swarm-spores of two kinds in *T. umbrina*, small biciliated gametes borne in hooked sporangia or gametangia and four-ciliated zoospores in globular zoosporangia. Whether or not the variations noted above in *Trentepohlia* sp. represent similar morphological differences is questionable, since the number of cilia cannot be observed when the zoospores are in the sporangia. However, in view of the fact that the normal swarm-spores appear somewhat larger and more oval after coming to rest, I doubt very much if this condition represents more than a difference in age and development.

The zoospores which fail to escape may either degenerate immediately, as noted before, or become actively motile for a considerable length of time. If the sporangium mouth becomes obstructed after a few of the zoospores have escaped, the others remain quiescent for a moment or two, then begin to move about slowly. They appear to be at first somewhat stuck together in the colloidal viscid substance in the sporangium, and a few seconds are required for complete separation. Within two minutes in one particular sporangium they were entirely free and separate and began moving about rather rapidly. The movement became accelerated, until in five minutes the zoospores were dashing wildly about as if completely frantic. Under these conditions zoospores may retain their motility considerably longer than under normal circumstances. Several sporangia have been very carefully studied in this respect. In one sporangium three zoospores were left behind and became very active within a few minutes. Two of these zoospores continued dashing about for forty minutes, while the third remained active for one hour and ten minutes, which is considerably longer than the motile period under normal conditions.

The zoospores vary in size and shape but are fairly uniform within certain limits. They are somewhat flattened and elongate, and when seen from the narrow side have the appearance shown in Pl. XVII, Fig. 44 *b*. In the other plane they are broader and have a tendency to be more ovoid, as seen in Pl. XVII, Figs. 44 *m*, 44 *o*, and 44 *q*. They none the less appear longer and less oval than those of *T. aurea*, *T. umbrina*, and *T. moniliformis*, which have been figured by Caspary, Karsten, and Meyer, and more closely similar to those of *T. lagenifera* as described by Wille (91). No very sharply defined plastids, such as have been figured by G. Karsten (42), are visible in the zoospores. They none the less appear green-granular, and this gives them their characteristic light green colour. The granules or droplets of chlorophyll seem, however, to be collected more or less in certain minute regions but are not sufficiently aggregated to present a definite plastid. The anterior and posterior ends are quite free from pigment and are hyaline in colour, as is shown in Pl. XVII, Figs. 37, 44, 45, and 46. No definite eyespot is visible. The two cilia are borne posteriorly, and appear to be of equal length and approximately two and a half times the length of the body. Zoospores with four cilia have also been observed, but very rarely. Whether or not this condition is always the result of fusion is uncertain from my material. In *T. umbrina*, as noted before, Meyer figures both types. The four-ciliated zoospores are vegetative and develop directly into new thalli, while the biciliated are regarded as gametes. Wille also described swarm-spores with four cilia which had been formed by unequal cleavage in the zoosporangium rather than by fusion.

The zoospores are plastic and undergo considerable change in shape under certain conditions. If one of these spores is caught in a tight place between the filaments or the slide and cover glass, it may elongate tremendously in attempting to get through, as shown in Pl. XVII, Fig. 44 *a*. It may become somewhat filamentous and take on the appearance of a short worm squirming through a narrow place. When once free, however, it assumes its characteristic shape and swims off with great rapidity. The movement of the swarm-spores is characteristic; it is essentially a back and forth darting movement rather than a slow, uniform forward swimming. The zoospores may dart about, come to rest, and then start off rapidly again in the opposite direction. Where zoospores were trapped in small confined areas they moved either backward or forward without turning about, showing that the cilia may propel the body readily in either direction. Hildebrand described a similar characteristic of the zoospores in *T. lagenifera* (Pl. XVII, Figs. 15 and 16).

In size the zoospores vary from  $4 \times 10 \mu$  to  $5 \times 14 \mu$ . The motile stage lasts in general from ten to thirty minutes but varies considerably with individual zoospores. As has been pointed out, in the case of motile spores which have failed to escape, the time is considerably longer. This



period is somewhat longer than that described by Wille for *T. umbrina*. Hildebrand, on the other hand, reported a motility of several hours in *T. lagenifera*. Towards the latter part of the motile period the zoospores tend to become more ovoid and less elongate in shape, as Caspary, G. Karsten, and Wille have shown. This was of universal occurrence in my material. Consequently when such spores are side by side with those which have recently escaped a marked difference is seen; this again may suggest heterogamy, but the differences are merely those of age. The tendency to become ovoid or globular becomes more pronounced as the spores come to rest, as shown in Pl. XVII, Figs. 44 *k*, 44 *n*, and 44 *p*. At this stage the posterior end, to which the cilia are attached, may be slightly beaked in shape. As the spores come to rest, the cilia are lost or drop off; they are not withdrawn into the spore body itself. The subsequent shape of the spores is characteristic and can be recognized readily in preparations of living material. They tend to be globose, ovoid, or slightly angular, as shown in Pl. XVII, Figs. 44 *d*, *e*, *f*, *j*, *h*, and *i*. At this stage the plastids are still indefinite, but the cytoplasm appears to be filled with a coarsely granular green pigment localized in small regions. Generally these granules or droplets are aggregated more towards the centre of the resting zoospore, leaving thus a somewhat narrow hyaline border at the periphery.

The majority of the thousands of zoospores which I have observed underwent disintegration shortly after coming to rest. This characteristic of *Trentepohlia* has been noted and emphasized by all students since zoospores were first discovered in this group of algae. Disintegration is usually accomplished by a vacuolation and gradual breaking up of the protoplasm. Generally Brownian movement soon begins and the entire resting zoospore becomes a mass of dancing fine particles. Within two to ten minutes the entire zoospore had disintegrated and diffused in the surrounding medium, leaving only numerous granules of various sorts scattered about.

As to conjugation of zoospores or gametes, I have observed but few cases. Throughout the past year thousands upon thousands of zoospores have been observed in hanging-drop and tap-water cultures, and up to the present only three cases of actual fusion have been observed. It is not at all uncommon, however, to find zoospores swimming in pairs or in threes or fours, as is shown in Text-figs. 8 and 9 and Pl. XVII, Figs. 45 and 46. Frequently zoospores are seen swimming in the same direction, giving the appearance of one following the other. They may come into contact, get their cilia entangled, and in this paired condition (Pl. XVII, Fig. 46) swim for some time. Careful observation of such pairs, however, has shown that they soon become disentangled, separate, and swim off independently (Text-fig. 8). In other cases three and sometimes four zoospores come out of the sporangium almost simultaneously with their cilia entangled and

swim off thus together; but these groups almost always separate after a short time. Pl. XVII, Fig. 45, and Text-fig. 9 show two groups of three and four zoospores together. These groups were carefully observed for some length of time, and the swarm-spores finally separated.

Oltmanns (69 *a*) regards the two types of sporangia which develop in *Trentepohlia* as markedly different qualitatively. The hooked sporangia (Hackensporangien) produce zoospores, while the globular ones (Kugelsporangien) are gametangia and form gametes. Oltmann's distinction was accepted by Meyer for *T. umbrina*. Heering likewise speaks of two kinds of zoosporangia, but questions the evidence for this differentiation. Printz (71) apparently regards the evidence as conclusive and distinguishes two types for the whole family Trentepohliaceae. Since I have rarely, if at all, found hooked sporangia, I am unable to confirm Oltmann's and Meyer's contentions, but I am certain that the motile spores from the globular sporangia may fuse or may germinate directly.

Stages in the actual process of fusion have been observed, and two cases of four-ciliated zoospores were found. However, it is not certain, in view of Wille's observations, whether such zoospores represent fusions or are normal four-ciliated zoospores. In *T. bleischii* Wille has described the delimitation of very large segments in cleavage which become four-ciliated and escape and swim about as normal zoospores. A similar large zoospore in *Trentepohlia* sp. is shown in Text-figs. 10 and 11 in its motile condition and also after coming to rest. The great difference in size between this body and that of normal zoospores is evident in this figure.

Up to the present time I have found only two cases of zoospore germination among the thousands of individuals studied. In coming to rest many of the zoospores lie very close together, quite frequently in pairs and threes, as is shown in Pl. XVII, Figs. 44 *g*, *h*, *i*, *j*, and *k*, and from such appearances one may be at first inclined to regard this as perhaps a stage in conjugation. For this reason very careful study has been made of the subsequent development of such pairs. The zoospores shown in *g*, *h*, *i*, and *k* were kept under observation until they had completely disintegrated, but no actual fusion between the resting spores occurred. The pair shown in *i* were at first thought to have fused, but by careful focusing it became apparent that a part of one overlapped the other, and there was no actual fusion. In *j* the contact is much closer, and it was difficult to make out a distinct line of separation between the two. Whether this represents a case of incomplete fusion is not at all certain. It may be nothing more than that two spores have come to rest in contact with each other, and in the process of disintegration their membranes have become less distinct. All of these spores disintegrated, and no further development occurred.

In Pl. XVII, Figs. 47 to 54, are shown the successive stages in germination of a zoospore. In Pl. XVII, Fig. 47, the zoospore has come to rest, is

oval in shape, slightly beaked at the point of attachment of the cilia, and hyaline at both ends. The green pigment is aggregated into fairly large granules or droplets disseminated throughout the body, except at the periphery. As yet no definite wall is present. Pl. XVII, Fig. 48, represents the same spore four hours later. The only conspicuous change is the presence of a definite membrane or wall around the spore. No further visible change appeared to occur until six hours later, when the resting spore began to elongate at what was formerly the posterior end (Pl. XVII, Fig. 50). This appears to be the first indication of germination. In the meantime, however, the pigment granules or droplets have become slightly deeper in colour and appear to be more definitely segregated. Pl. XVII, Fig. 51, shows a stage three hours later in which cell-division has occurred. The newly-formed daughter-cell is slightly smaller and more slender in shape. Pl. XVII, Fig. 52, shows the same zoospore a day later. A short thin segment consisting of four cells has been formed, in which the limits of the plastids are becoming more distinct. In addition minute highly refractive globules are present in the cytoplasm. These are distinctly hyaline, and do not have the golden or yellowish tinge that becomes characteristic later. Pl. XVII, Fig. 53, shows a further developmental stage in which the apical cell of the filament has elongated tremendously and is doubtless ready to divide. The plastids are now definitely visible and delimited as single entities much deeper green in colour, with a tendency to lie in the primordial utricle of the cell. The refractive hyaline globules are much larger in size and more numerous.

The development of the plastids in the germinating zoospore appears thus to be fairly distinct, and can be followed with some degree of certainty. At first the pigment appears to be distributed throughout the cytoplasm in certain small areas in the form of droplets or granules, and no visibly differentiated or delimited plastids are visible. As germination proceeds, however, the pigment takes on a deeper colour, and the regions in which it is distributed become more definite and microscopically visible until finally discrete plastids with apparent membranes are present.

A later stage in germination of this particular zoospore is shown in Pl. XVII, Fig. 54. The basal, second, and fourth cells of the filament have produced typical branches. Further developmental stages of this particular spore were not observed, but a few young thalli such as shown in Pl. XVI, Fig. 17, have been found separately which were probably more advanced stages of germination. One case of germination *in situ* has been found, and is illustrated in Pl. XVII, Fig. 55. The sporangium here shown contains four zoospores, three of which have rounded up and become invested with a definite membrane, while the fourth has undergone germination to produce a short filament consisting of three cells. The apical end of this filament is directed toward the opening of the sporangium, but subsequent

developmental stages were not followed to determine whether or not it grew out at this end.

#### GENERAL MORPHOLOGY, DEVELOPMENT, AND REPRODUCTION OF THE FUNGUS.

The fungus constituent *Coenogonium Linkii* has not been studied extensively except in its mature reproductive stages, although the type, size, and shape of the ascospores as well as the number, septation, and size of the ascospores are generally used as criteria in classifying the lichen species. It has not, so far as I am aware, yet been named or assigned independently to any particular family and genus of fungi outside of the lichens. Mycologists who treat the lichens at all generally include them with the fungi and retain the lichen name for the fungus as well, since in the majority of forms it is the predominant constituent of the alliance, and forms the conspicuous fruiting structures. In line with this general practice Clements (8) and Clements and Shear (9) retain the name *Coenogonium* for the fungus, and place it in the family Chrysotrichaceae of the order Pezizales.

The vegetative thallus consists of a septate, branched, irregular mycelium which anastomoses abundantly and forms a fairly dense web or net around the algal filaments. The mycelium varies in diameter, and is usually quite uneven in contour. Small papillae or buds stand out from the surface and produce the characteristic irregular appearance shown in Pl. XVI, Fig. 10, and Text-fig. 12. It is usually hyaline in colour, but at the base of the lichen thallus is often slightly brown and olivaceous. The branches may run out for considerable distances from the algal filaments, and when they come into contact with other branches or filaments of the alga they become closely appressed or attached, as is shown in Pl. XVI, Fig. 17. This type of branching and method of attachment are doubtless the means by which the fungus gets from one algal filament to another, and makes the rather dense web of mycelium surrounding several filaments in the older portions of the lichen thallus, as is shown in Text-fig. 12.

As has been noted before, no haustoria are formed, and the alga does not appear to suffer materially from its association with the fungus. The latter, however, seems in some manner to be very dependent on *Trentepohlia*, since it has not yet been found growing independently. Many attempts have been made by the writer to germinate ascospores on corn meal, potato, Detmer's agar, and prune agar, and also to transplant portions of the mycelium, but without apparent success. On Detmer's agar, the alga grows fairly well, but the fungus dies and disappears in a few weeks.

Frequently large dark phragmosporous and dictyosporous conidia are attached to the alga in great abundance, as is shown in Text-fig. 13, but I am uncertain as to whether or not these spores belong to the same fungus

species. Frequently when they germinate their germ-tubes and mycelium parallel the algal filament, as in Text-fig. 13. The mycelium is similar in diameter, septation, and habit of branching, but much darker in colour. The germinating spore shown in this figure is similar to those figured by Bornet in *Biatora muscorum* where they are without question a part of the lichen. I have isolated several of these large spores, and cultured them readily on various agar media. They produce an abundant mycelium, but so far no fruiting bodies have developed which would enable me to identify the species. The ease with which they germinate and their vigorous growth on synthetic media are in sharp contrast to the ascospore germination noted above; and for this reason I am at present very doubtful that the two are the same fungus. In addition to these large spores numerous elongated, plurilocular, brown and olivaceous insect eggs are present in the lichen thallus, and may be readily mistaken for fungus spores.

The fungus reproduces asexually by small conidia produced in globular pycnidia, as has been figured by Nylander, and described by a number of other workers on *Coenogonium*. Nylander's figure of a pycnidium is small, and gives no details of internal structure. The pycnidia appear to be borne on the lichen thallus in the same manner as the discocarps, and frequently include in their outer stroma, as is shown in Pl. XVIII, Fig. 56, filaments of the alga. The early developmental stages are difficult to distinguish from the very young round apothecia, and it is only by study of successive stages that the two can be differentiated. As the pycnidium grows in size, it spreads so as to cover many of the alga filaments.

Pl. XVIII, Fig. 56, shows a longitudinal section of the pycnidium from fixed and stained material. The pycnidium is markedly globular in shape, without a prominent beak. The ostiole is usually only slightly elevated. The wall is fairly thick, and consists of dense and compact layers of short hyphae which often give the appearance of pseudoparenchyma. It does not appear to have any markedly differentiated layers, since the outer portions gradually merge into the sporogenous layer. The outer hyphae, however, are less dense in nuclei and cytoplasm, and thus appear more hyaline. The conidia are borne on fairly short conidiophores, although the minute size of the latter and the myriads of spores make it very difficult to observe accurately the exact size and structure in fixed and stained preparations. The conidia are elongate, tapering, hyaline, continuous, uninucleate, and vary from  $5 \times 2 \mu$  to  $8 \times 1 \mu$ . They are frequently found lying end to end or closely adjacent in the pycnidium, and at first sight seem to be catenulate, but it has been impossible to determine this with certainty. The spores are borne around the entire inner periphery of the pycnidium, but become fewer toward the ostiolar region. Pl. XVIII, Figs. 57 to 60, show stages in the formation of the conidia and their variation in size and shape. The young conidiophores are slightly club-shaped at their

upper end (Fig. 57), and by successive growth and constriction the conidia are finally delimited, as shown in Figs. 58 and 59. The conidiophores in these figures have nuclei in their basal portions, which suggests that they are able to form more than one conidium. This is further substantiated by the occurrence of elongated conidiophores which show several nuclei and constrictions (Fig. 56). The nuclei of the conidia are rather conspicuous in fixed and stained preparations, and usually lie in the centre in an isthmus of cytoplasm, while the remainder of the cell is highly vacuolated.

In addition to the conidia, numerous very fine hyaline hairs or mycelial strands ramify among the spores in the pycnidium, as shown in Pl. XVIII, Figs. 56 and 61. Nuclei are distributed more or less regularly along their length, but because of their minute size and the myriads of conidia it is impossible in my preparations to determine whether septa are present. These filaments, which often extend to the ostiole, resemble paraphyses very closely, and appear to originate in the sporogenous layer in the same manner as the conidia. They are hardly to be regarded, it seems to me, as stylo- or scoleospores.

The apothecia or discocarps occur, as noted before, in great abundance on the under side of the lichen thallus (Pl. XVI, Fig. 2) and are waxy and light yellow to light orange in colour. In my material they vary considerably, 0.3 to 0.8 mm., in diameter at the apex, as shown in Pl. XVI, Figs. 3 and 4. These are both mature apothecia and not successive stages in development. The two apothecia here figured are rather flat on top, and are hardly representative in this respect. The ascogenous layer is usually more elevated in the centre, and tapers off towards the periphery, so that the upper surface is more convex, as has been shown by H. Karsten and Schwendener. The stalk or stipe of the discocarps varies both in shape and length. It may be very short and thick as in Pl. XVI, Figs. 3, 4, and 9, or somewhat elongated and curved, Pl. XVIII, Fig. 62.

Numerous developmental stages such as are shown in Pl. XVI, Figs. 5 to 8 have been found. The earliest stages consist of a whorl or snarl of short mycelial branches which, with subsequent growth, become more and more numerous and entangled. Fig. 5 shows a very early stage with four short irregular branches which have come into contact at their apices, and are beginning to intertwine. This figure is closely similar to that of an early stage illustrated by Johow (39, Fig. 36) in material from Dominica. On the opposite side of the algal filament is a short branch which has become completely overgrown by the fungus, but this is not in my opinion the rudiment of an apothecium. Karsten, Schwendener, and Nylander (67) describe and figure the apothecia as originating around and over the short algal branches, but this is not universal. In my material they have been found occurring at the end and on the sides of *Trentepohlia* filaments as well as around the short branches. Pl. XVI, Fig. 6, shows a slightly later

stage in which the hyphal branches are more numerous and densely intertwined. In the initial stages the young apothecia are usually confined to one or a few algal filaments, but as they grow in size they spread and involve a large number. Doubtless the fungus hyphae around these additional filaments are included and aid in building the ascocarp. The apothecium shown in Pl. XVI, Fig. 7, has grown to considerable size, and has become definitely spherical. The inner hyphae appear to make a more or less compact weft, while the outer ones are still loose. A later stage is shown in Pl. XVI, Fig. 8, in which the hyphae are more compact and form a fairly round pseudoparenchymatous mass. The algal filament has been almost completely enveloped by the young apothecium, and this growth doubtless accounts for the inclusion of several filaments in the basal stroma of mature apothecia.

The subsequent stages of development consist essentially of an increase in size of the spherical mass and slight elongation to differentiate the stipe and ascogenous layer. In none of the fixed and stained preparations have trichogynes or ascogenous hyphae so far been observed. My observations and data on the late stages of apothecial development are rather meagre, and it is thus impossible to describe with any degree of accuracy the origin and development of the ascogenous layer. A further study on the structure and development of the apothecia is now in progress, and will be reported in a later paper.

A mature discocarp is shown under higher magnification in Pl. XVIII, Fig. 62. The stalk or stipe is very small in comparison with the upper portion, and in this respect is rather exceptional. The asci and paraphyses make a very dense and compact layer, and are rather difficult to differentiate individually in fixed and stained material. In living material they may be readily dissected out and studied. Pl. XVIII, Fig. 63, shows several asci and paraphyses under high magnification. The former are cylindrical, somewhat blunt at the apex, taper at the base, and vary  $5 \times 80$  to  $5 \times 40 \mu$ . The paraphyses are inflated, septate and branched at the apex, and usually a little longer than the asci. The ascospores are two-celled, vary from  $1.5 \times 5$  to  $3 \times 6 \mu$ , and, as is shown in Pl. XVIII, Fig. 64, may be ovoid or fusiform in shape. The ovoid shapes are exceptional, and the majority of spores are markedly fusiform.

#### SUMMARY.

1. *Coenogonium Linkii* has been collected in damp regions on saplings, twigs, branches, vines, and the bark of larger trees in Southern Mexico, British Honduras, Guatemala, and Honduras.
2. The thallus consists of branched, more or less parallel filaments which are united to form flat, somewhat saucer-shaped, chlorophyll-green,

bracket-like felts, 3 to 20 mm. in diameter with concentric zones. Light yellow to light orange waxy apothecia are borne in abundance on the under side of the thallus. The filaments which compose the thallus consist of a central row of large cylindrical *Trentepohlia* cells and numerous peripheral, hyaline, septate mycelia which branch and anastomose to form a net-like structure around the central row.

3. *Trentepohlia* sp., the gonidium of this lichen, is made up of branched septate filaments with cylindrical cells varying from  $10 \times 10 \mu$  to  $38 \times 6 \mu$ . Numerous oval, elongated, somewhat irregular and band-shaped plastids, together with large numbers of round, hyaline, refractive, yellow and orange oil globules or haematochrome bodies of various sizes, are present in the cytoplasm. Considerable variation in the shape and size of the thalli, individual cells, and sporangia occurs when this alga is grown in culture.

4. *Trentepohlia* sp. reproduces by the growth of isolated vegetative cells, aplanospores, zoospores, and gametes. The zoosporangia are terminal, lateral, or intercalary, sessile or stalked, and round, ovoid, or egg-shaped. None of the so-called hook- or 'trichter'-sporangia were found either in nature or agar cultures.

5. The zoospores and gametes are delimited in the zoosporangia by progressive cleavage. They are elongate and somewhat flattened in shape, and vary from  $4 \times 10 \mu$  to  $5 \times 17$  with two posteriorly attached cilia. They may fuse or germinate, forming new thalli directly. The majority of zoospores disintegrate shortly after coming to rest; only three cases of fusion and two of germination have been observed.

6. The fungus constituent of *C. Linkii* consists of septate, hyaline, branched, irregular mycelium with branches which frequently anastomose and form a dense net or web around the algal filaments. It grows closely applied to the alga, but does not become intracellular. No haustoria have been observed.

7. The fungus appears to be dependent on the alga for continued growth, but apparently does not injure it. Efforts to culture the mycelium, and germinate the ascospores and conidia have been unsuccessful.

8. Reproduction occurs by conidia and ascospores. The conidia are hyaline, continuous, elongate, fusiform, uninucleate, vary from  $1 \times 5 \mu$  to  $2 \times 8 \mu$ , and are borne in round pycnidia.

9. The discocarps occur in great abundance on the under side of the thallus, and vary from 0.3 to 0.8 mm. in diameter at the apex. They originate from small whorls or snarls of hyphae which grow in size, become spherical, and later elongate and differentiate into the mature apothecia.

10. The ascospores are elongated and fusiform, two-celled, hyaline, and  $1.5 \times 5 \mu$  to  $3 \times 6 \mu$  in size. The paraphyses are inflated, septate and branched at the apex, and usually longer than the asci.



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## EXPLANATION OF PLATES XVI to XVIII.

Illustrating Dr. John S. Karling's paper on 'A Preliminary Contribution to the Structure and Development of *Coenogonium Linkii*'.

(All drawings were made from living material of *C. Linkii* with the exception of figures 9, 56, 57, 58, 59, 60, 61, 62, 63, and 64.

## PLATE XVI.

- Fig. 1. Portion of a twig with three lichen thalli.  
 Fig. 2. Underside of a thallus showing the distribution of the apothecia.  
 Figs. 3 and 4. Mature apothecia.  
 Fig. 5. An early stage in the development of the apothecium. On the opposite side is a short branch of the algal filament completely overgrown by the fungus.  
 Fig. 6. A later stage in apothecial development.  
 Fig. 7. A young apothecial initial composed of loosely united hyphae.  
 Fig. 8. A slightly older apothecium.  
 Fig. 9. Longitudinal section of a mature apothecium.  
 Fig. 10. A portion of the algal constituent (*Trentepohlia* sp.), showing the size, shape, and contents of the cell, method of branching, and relation to the fungus constituent.  
 Figs. 11 and 12. Surface views of ends of the algal filaments showing the relation of the fungus to the alga.  
 Fig. 13. Variations in size and shape of plastids of *Trentepohlia* sp.  
 Fig. 14. Variations in size and shape of the refractive globules or haematochrome bodies in *Trentepohlia* sp.  
 Fig. 15. Filament of *Trentepohlia* sp. from Detmer's agar cultures showing the abundance of zoösporangia and various stages in development.  
 Fig. 16. A young isolated germinating vegetative cell, or possibly a zoospore of *Trentepohlia* sp.  
 Fig. 17. A young sporeling showing the manner of attachment of the fungus.  
 Fig. 18. Cross section of a short branch of *Trentepohlia* sp. showing the abundant development of the fungus in such regions.  
 Fig. 19. Cross section of *Trentepohlia* sp. showing the number and relation of fungus hyphae.  
 Fig. 19a. Portion of a filament of *Trentepohlia* sp. with isodiametric cells from near the base of the lichen thallus.

## PLATE XVII.

- Fig. 20. A young thallus of *Trentepohlia* sp. from agar cultures.  
 Fig. 20a. Zoospores escaping from a sporangium.  
 Fig. 21. A young thallus of *Trentepohlia* sp. with moniliform cells.  
 Fig. 22. Terminal portion of a thallus of *Trentepohlia* sp. in which all but the apical cell and short lateral branches have died.  
 Fig. 23. Two intercalary cells of *Trentepohlia* sp. showing the phenomenon of 'Durchwachsung'.  
 Fig. 24. Intercalary aplanospore of *Trentepohlia* sp.  
 Figs. 25, 26, 27, and 28. Isolated and germinating vegetative cells of *Trentepohlia* sp.  
 Figs. 29 and 30. Variations in size and shape of zoösporangia of *Trentepohlia* sp. in agar cultures.  
 Fig. 31. Stalked zoösporangium enveloped by fungus hyphae.  
 Fig. 32. Sessile, lateral, vacuolated zoösporangium.  
 Figs. 33, 34, and 35. Early, later, and completed stages in cleavage respectively.  
 Fig. 36. Zoösporangium with mature zoospores. The basal cell has divided and is continuing growth.  
 Fig. 37. Escape of the zoospores.  
 Fig. 38. Zoösporangium in which the zoospores have failed to escape and become larger and more rounded.

Fig. 39. Young lateral zoosporangium separated from its basal cell.

Fig. 40. Isolated zoosporangium with ovoid zoospores which have come to rest within.

Figs. 41 and 42. Beaks of the sporangia before and after opening.

Fig. 43. Empty, collapsed zoosporangium with three quiescent zoospores within.

Fig. 44 *b*. Zoospore viewed from the narrow side.

Fig. 44 *o* and *m*. Zoospores viewed from the broad side.

Fig. 44 *a*. Zoospore drawn out and elongated in passing through a narrow opening.

Figs. 44 *k*, *n*, and *p*. Changes in shape of zoospores at the end of the swarm period and shortly before coming to rest.

Figs. 44 *d*, *e*, *f*, *h*, *j*, and *l*. Differences in size and shape of the zoospores shortly after coming to rest.

Figs. 44 *g*, *i*, *k*. Resting zoospores, in pairs.

Fig. 45. Group of three zoospores stuck together.

Fig. 46. Two zoospores with cilia entangled.

Figs. 47 to 54. Successive stages in the germination of a zoospore.

Fig. 55. Germination *in situ*.

#### PLATE XVIII.

Fig. 56. Mature pycnidium of fungus.

Figs. 57 to 59. Stages in the formation of conidia.

Fig. 60. Variations in size and shape of conidia.

Fig. 61. Hair or filament from pycnidium.

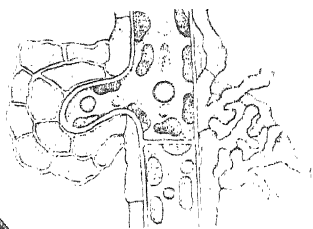
Fig. 62. Longitudinal section of mature apothecium.

Fig. 63. Asci and paraphyses.

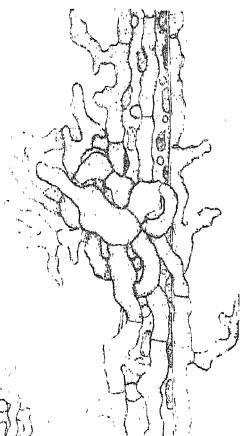
Fig. 64. Variations in size and shape of ascospores.



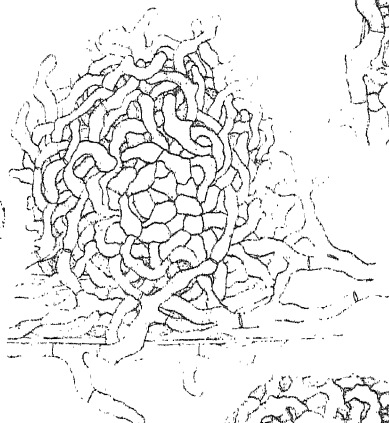
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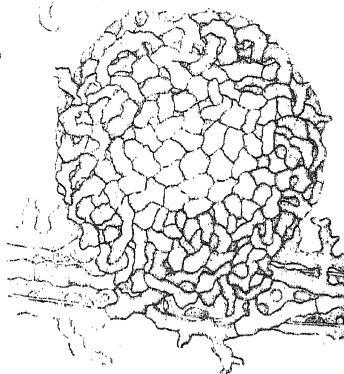
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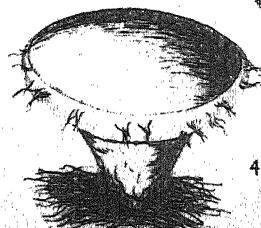
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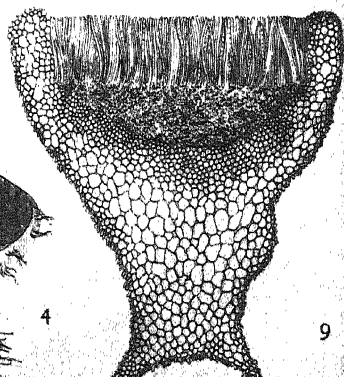
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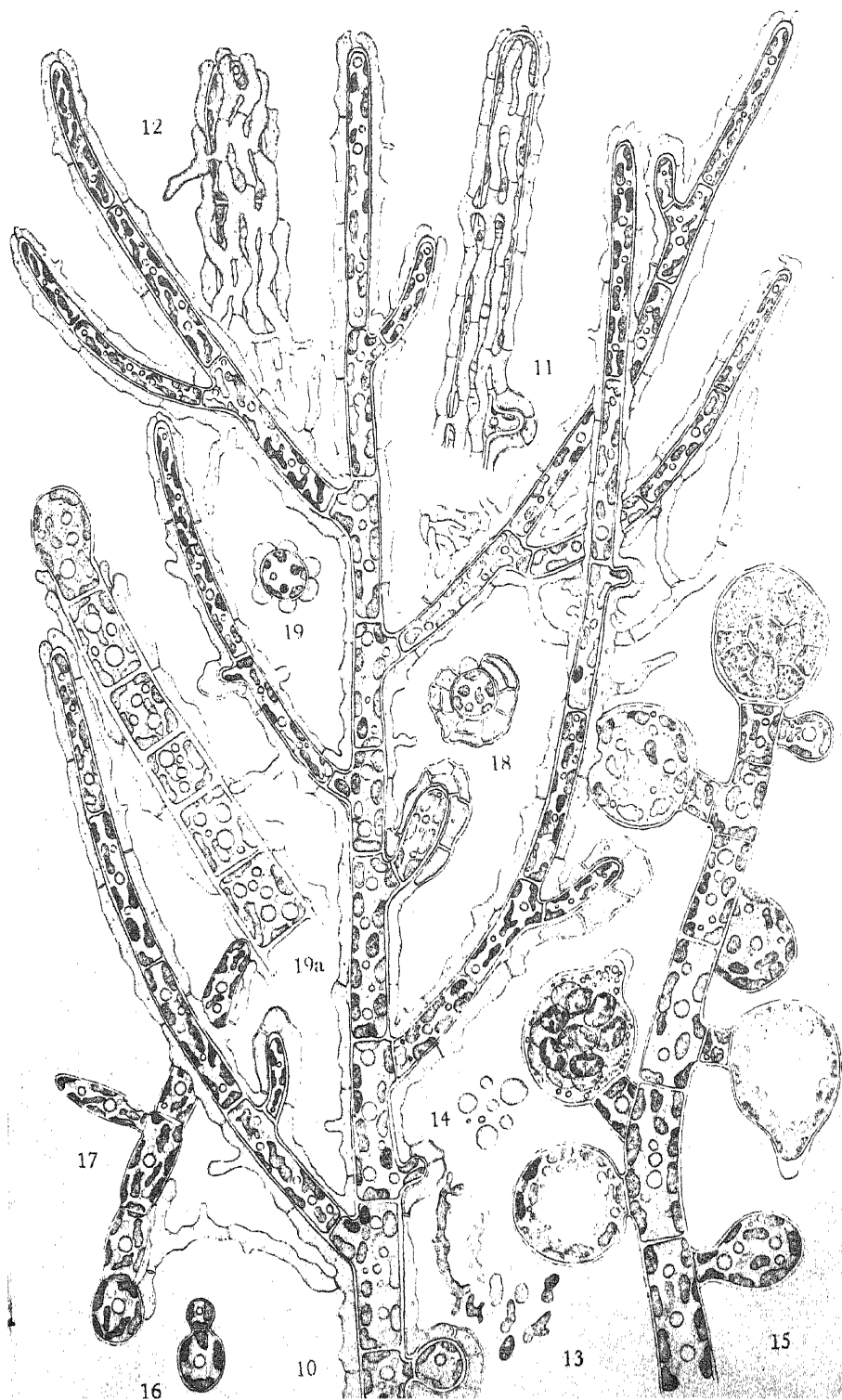
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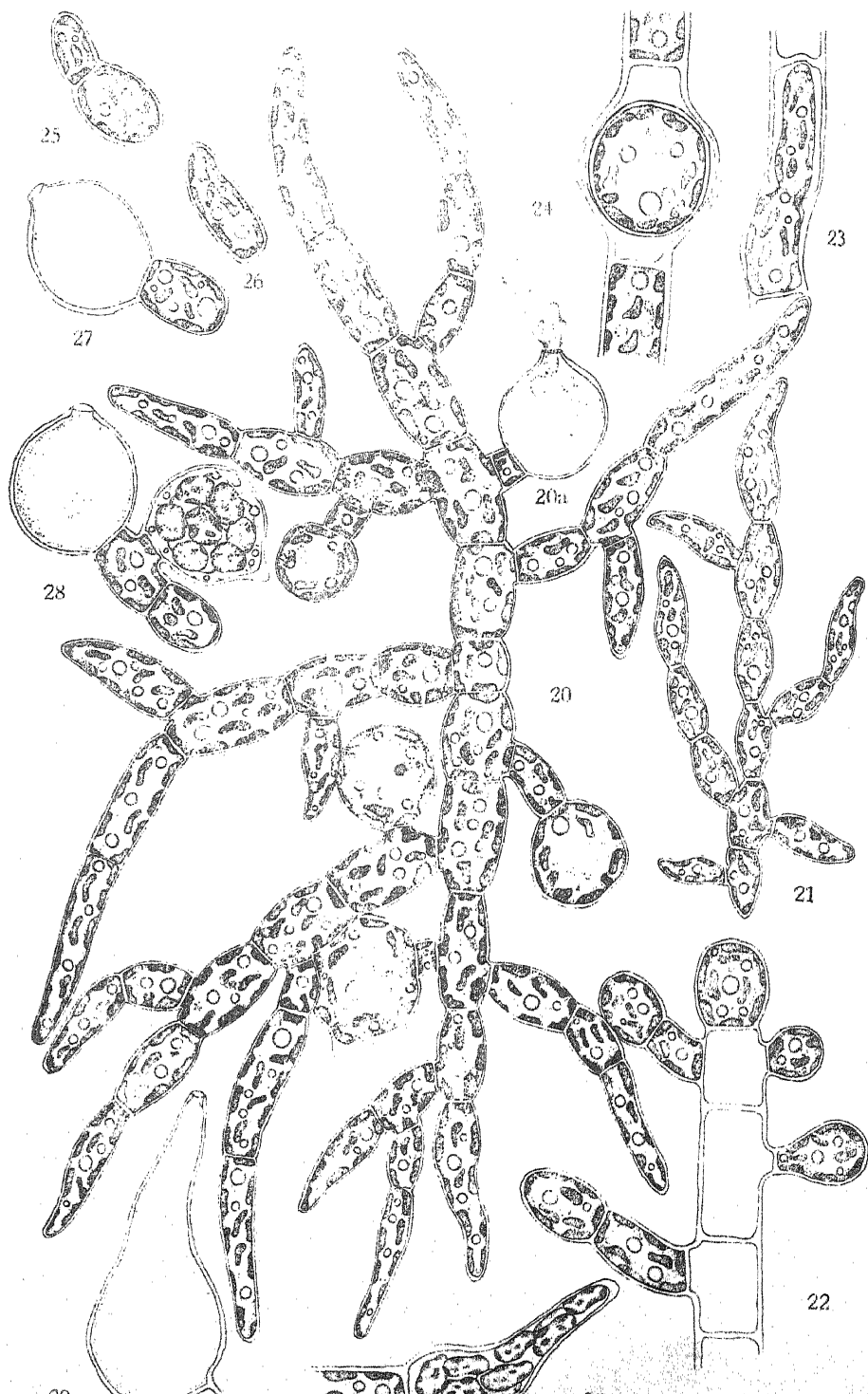


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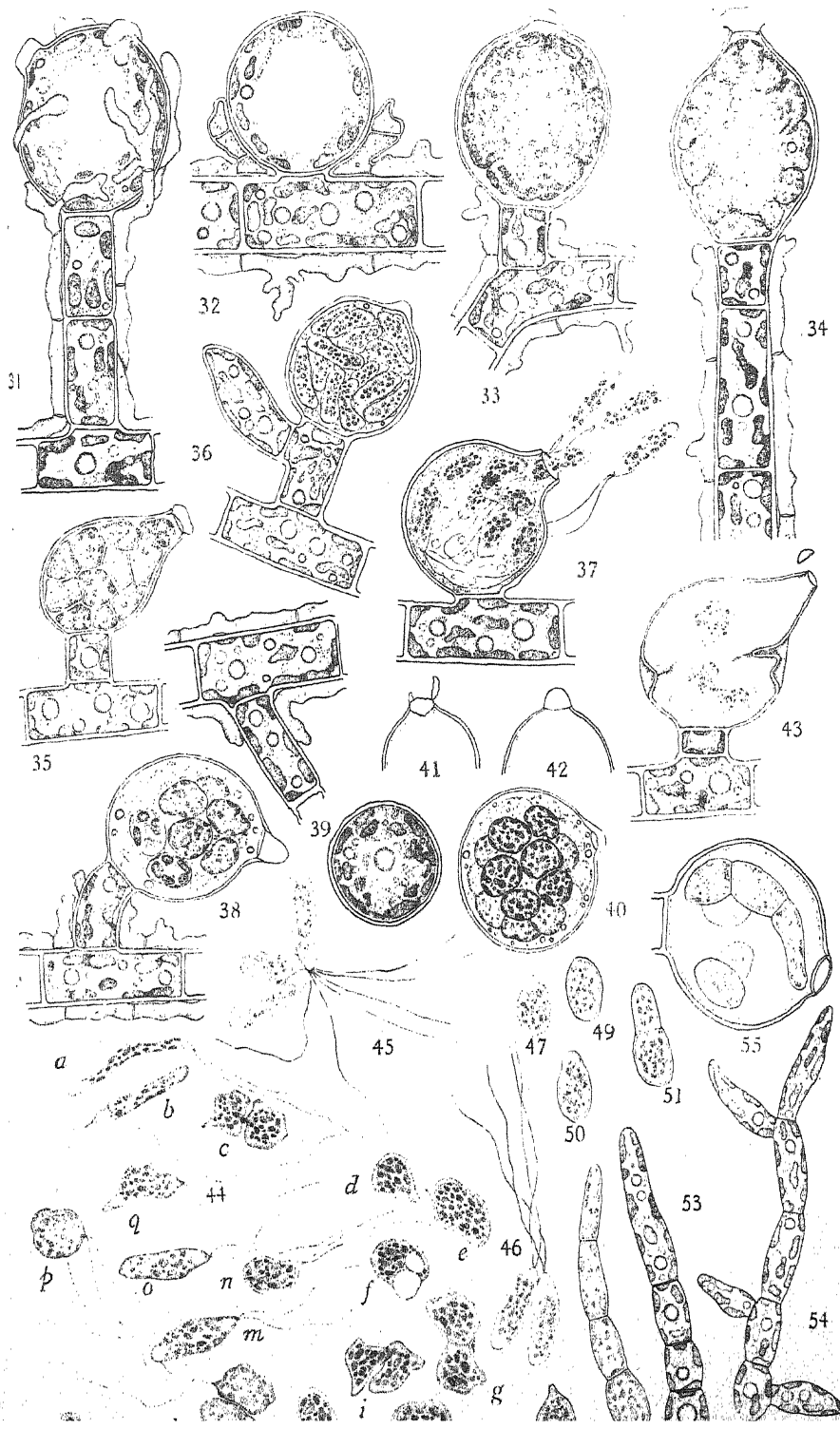


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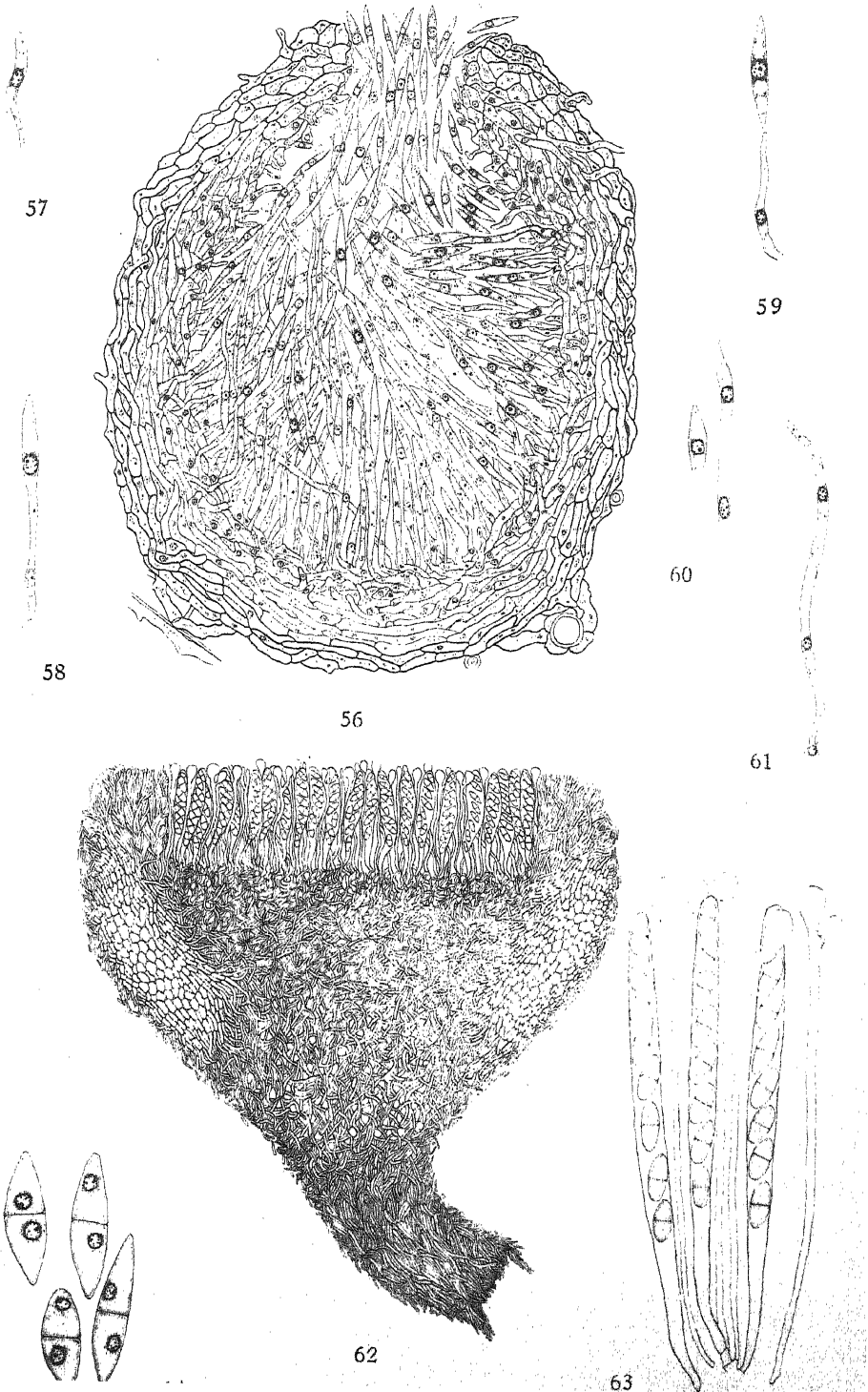














# Seasonal Wounding and Resin-cyst Production in the Hemlock, *Tsuga canadensis* (L.) Carr.

BY

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With Plate XIX and four Figures in the Text.

## INTRODUCTION.

THE production of resin tissue in response to wounding is characteristic of certain conifers, and in some groups, such as the Abietae, the relationship is more evident than in others. To determine the character of this response to wounding at different seasons of the year, studies were made with *Tsuga canadensis*, a member of this group. The preliminary work of wounding was done by Professors R. B. Thomson and H. B. Sifton, who handed the material over to the writer for detailed examination. Branches of similar size occupying the same relative positions on the lower parts of old trees were selected, and wounded throughout the year with shorter intervals during the growing season. Half-round  $\frac{1}{8}$ - and  $\frac{3}{16}$ -inch chisels were used. Usually two, in some cases more, branches were wounded on each date, one with each chisel. Six to eight wounds, spaced at intervals of 23 cm., were made on the upper side of each branch. For each wound two contiguous cuts were made, so that an elliptical area of bark and cambium with a minor diameter of  $\frac{1}{8}$  or  $\frac{3}{16}$  inches and a slightly greater major diameter (lengthwise of the branch) was removed. Four stems of young trees were also wounded. After one or two years the wounded material was collected.

The method employed in determining the approximate extent of the resin tissue about each wound was to cut transverse sections at the wound centre and at centimetre distances above and below. For more detailed study these were supplemented by series of transverse or tangential sections. In few cases was it found necessary to embed the material since suitable sections could readily be cut with a sliding microtome.

The response to wounding usually consists of the formation of

a parenchymatous sheet of tissue, broken at various points by short and irregularly shaped cavities. These, owing to their shape and content, have been termed resin cysts (6). In making comparison of the experimental results, either the total extent of the parenchymatous tissue or the number of resin cysts might have been used, but as the resin cyst is the ultimate form of traumatic expression it was decided to make this the unit of comparison. Accordingly, the number of resin cysts at a given wound is taken as the index of its response, and the results of the wounding at various times of the year compared on this basis.

#### EXPERIMENTAL RESULTS.

The results obtained from each of the thirty-one wounded branches and stems are summarized in Tables I, II, and III. Under the heading 'age' at the left the number of annual rings in the branch or stem at each point of wounding is given. In Table I the maximum number of cysts in one section at or near each wound (usually 1 cm. above) is shown, and in Table II the average number at four points—at the wound, 1 cm. below, and 2 cm. and 1 cm. above. The results of the stem wounding may be seen in Table III.

In Tables I and II it is to be noted that the greatest production of resin cysts is in branches numbered 58, 59, 60, 63, 1, 2, and 3 which were wounded during June and July. Even in branch 60, which has the smallest resin-cyst production of these seven branches, there are more cysts than in the branches wounded at other times of the year.

In the stems (Table III) the greatest responses are in 61 and 66, wounded in June and August. There are few cysts in stems 50 and 55, wounded during April and May. Accordingly, the greatest development of resin tissue is in response to the wounds of the late spring and early summer period, as is the case in the branches.

A study of the wounded branches shows that cambial activity, as manifest by the production of tracheids, began between May 24 and June 15 and ceased prior to August 12. The wounding of the latter date stimulated the cambium to further activity, and a few tracheids were cut off in the vicinity of the wounds but not elsewhere. The normal growth period was thus limited to June, July, and possibly the first week in August. Since the greatest production of resin tissue was in the branches wounded during the growth period, there is a definite relationship between the amount of response and cambial activity.

It is worthy of note that the period of growth in the wounded branches was of short duration. As these branches were on the lower parts of the trees and were almost continually shaded the conditions were unfavourable to growth, a feature to which Brown (2) has called attention.

TABLE I. *Wounded Branches.*

Age in years.	74.	75.	10.	11.	12.	13.	14.	15.	17.	51.	52.	53.	56.	57.	58.	59.	60.	63.	1.	2.	3.	67.	68.	69.	70.	72.	73.
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	30	—	—	—	—	—	—	—	—	
5	—	—	—	—	—	—	—	5	5	—	—	—	—	—	—	—	—	21	15	—	—	—	—	—	—	—	
6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	40	—	—	—	—	—	—	—	—	
7	—	—	—	—	—	—	—	—	7	—	—	—	—	—	70	—	14	—	—	—	—	—	—	—	—	—	
8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	60	—	—	—	—	—	—	—	—	—	—	—	
9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	80	—	38	40	25	40	—	—	—	—	—	—	
10	—	—	—	—	*30	14	3	*33	50	—	—	—	—	—	90	65	17	46	—	50	—	—	—	—	—	8	
11	—	—	—	—	—	—	—	—	14	—	—	—	—	—	52	40	24	30	—	—	—	—	—	—	—	—	
12	—	—	—	—	—	—	—	—	20	—	—	—	—	—	52	40	—	30	—	—	—	—	—	—	—	—	
13	—	—	—	—	—	—	—	—	12	—	—	—	—	—	70	35	—	46	—	75	—	—	—	—	—	—	
14	—	—	—	—	—	—	—	—	6	—	—	—	—	—	70	38	—	—	—	—	—	—	—	—	—	—	
15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
17	—	—	—	—	*15	3	5	6	—	—	—	—	2	—	—	—	17	—	95	50	115	—	—	—	—	—	
18	—	—	—	—	2	11	2	—	—	3	1	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	
19	—	—	—	—	0	—	—	—	—	3	5	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	
20	—	—	—	—	6	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
21	—	—	—	—	—	—	—	—	—	9	4	3	1	3	—	—	—	—	75	—	70	6	—	—	—	—	
22	—	—	—	—	—	—	—	—	—	11	7	4	14	21	—	—	45	—	52	—	65	—	—	—	—	*42	
23	—	—	—	—	—	—	—	—	—	18	7	4	2	27	—	—	—	—	60	—	—	—	—	—	—	—	
24	—	—	—	—	—	—	—	—	—	12	3	2	20	—	—	—	21	—	45	—	—	—	—	—	—	*75	
25	—	—	—	—	—	—	—	—	—	4	—	—	9	—	—	—	18	—	—	—	—	—	—	—	—	—	
26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
31	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Aver- age.	3	0	3	3	2	9	3	6	17	9	4	3	6	16	68	41	24	35	58	56	80	6	4	2	6	3	5

\* The figures show the maximum number of cysts in a single transverse section at or near each wound.

TABLE II. *Wounded Branches.*

Age in years.	74.	75.	10.	11.	12.	13.	14.	15.	17.	51.	52.	53.	56.	57.	58.	59.	60.	63.	1.	2.	3.	67.	68.	69.	70.	72.	73.
4	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	11	—	—	—	—	—	—	—	—	Dec. 8.
5	—	—	—	—	—	—	—	3	4	—	—	—	—	—	—	6	—	16	—	—	—	—	—	—	—	—	Dec. 8.
6	—	—	—	—	—	—	—	21	5	—	—	—	—	—	20	—	20	20	10	—	—	—	—	—	—	—	Nov. 3.
7	—	—	—	—	—	—	—	21	22	—	—	—	—	—	27	18	7	14	—	—	—	—	—	—	—	—	Sept. 22.
8	—	—	—	—	—	—	—	21	5	—	—	—	—	—	36	28	14	18	10	—	—	—	—	—	—	—	Sept. 22.
9	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	Aug. 12.
10	—	—	—	—	—	—	—	21	5	—	—	—	—	—	29	16	14	18	10	—	—	—	—	—	—	—	July 17.
11	—	—	—	—	—	—	—	21	22	—	—	—	—	—	36	28	8	18	32	18	—	—	—	—	—	—	July 17.
12	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
13	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
14	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
15	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
16	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
17	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
18	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
19	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
20	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
21	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
22	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
23	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
24	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
25	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
26	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
27	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
28	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
29	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
30	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
31	—	—	—	—	—	—	—	21	22	—	—	—	—	—	38	28	8	18	32	18	—	—	—	—	—	—	July 17.
Average.	1	0	1	1	1	3	1	2	8	3	1	1	2	10	32	18	10	15	40	33	41	2	1	1	2	1	2

\* Average number of resin cysts at 2 cm. and 1 cm. above the wound centre, at the wound centre and 1 cm. below each wound.



If the growing season were longer, it is probable that the time of extensive resin-tissue production would have been correspondingly lengthened. Indications of this are found in the wounded stems. Here growth continued later in the season, and more resin tissue resulted from the wounding of August 12 than in the branches.

TABLE III.  
*Wounded Stems.*

	50.	55.	61.	66.		50.	55.	61.	66.
Age in years.	Apr. 13.	May 12.	June 30.	Aug. 12.		Apr. 13.	May 12.	June 30.	Aug. 12.
3	—	—	50	—		—	—	37	—
4	—	30	120	—		—	21	78	—
5	—	4	60	—		—	2	33	—
6	—	5	50	—		—	2	45	—
	—	10	—	—		—	4	—	—
7	—	10	40	17		—	6	29	5
8	—	3	—	—		—	1	—	—
9	—	—	—	—		—	—	—	—
10	6	—	—	—		2	—	—	—
11	5	—	—	35		2	—	—	22
12	—	—	—	—		—	—	—	—
13	6	—	—	—		2	—	—	—
14	—	—	—	—		—	—	—	—
15	—	—	—	55		—	—	—	19
16	—	—	—	—		—	—	—	—
17	7	—	—	—		2	—	—	—
28	—	—	—	35		—	—	—	13
Aver- age.	6	10	64	36		2	6	44	15

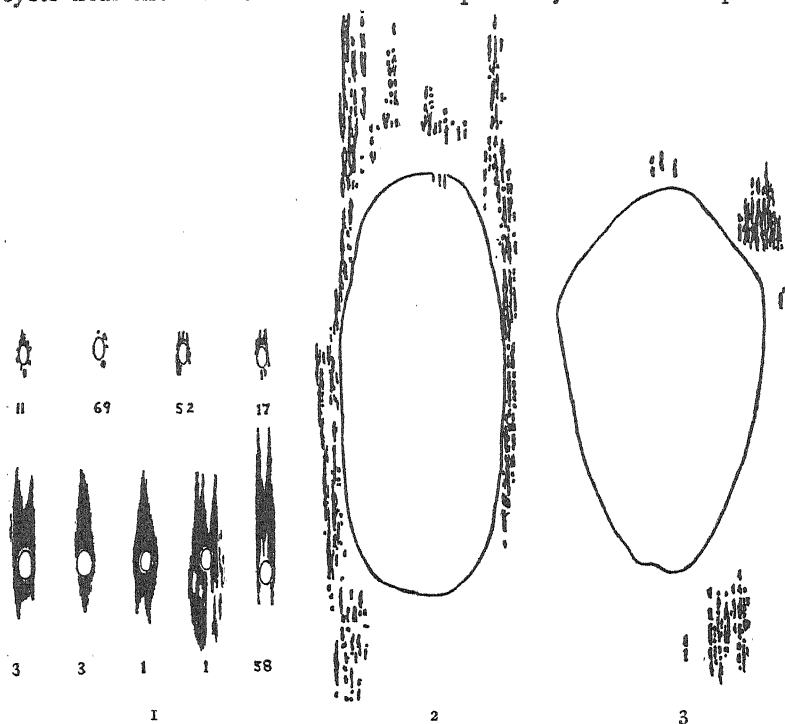
Maximum number of cysts per wound.

Average number of cysts at distances  
+2 cm., +1 cm., 0 cm., and -1 cm.  
from wound.

Wounds inflicted during the dormant period show meagre responses; resin cysts are few in number and usually limited in extent. This is illustrated in the upper row of Text-fig. 1 and also in Text-figs. 2 and 3. In Text-fig. 3 there are two small localized groups of cysts. The cysts are more numerous and widely spread about the wound shown in Text-fig. 2, being found as far as 0.7 cm. above and 0.6 cm. below the wound centre—but are still far from abundant. The parenchyma cells constituting the tangential sheet of tissue in which the cysts are embedded extend in some cases as far as 1 cm. above or below wounds (Pl. XIX, Figs. 1 and 8), but, more often die out and are replaced by tracheids at that distance (Pl. XIX, Figs. 6 and 7).

In the case of the wounds of the pre-growth period the resin cysts are

in the earliest spring wood adjacent to the preceding summer wood. The cysts are usually in a single tangential series, but where there are only a few cysts near the wound and the zone of parenchyma tissue is quite wide



TEXT-FIGS. 1-3. 1. The upper row shows the approximate extent of resin cysts at four dormant period wounds and the lower that at five growing period wounds. The numbers of the branches are given below each wound. 2. Details of the distribution of resin cysts at wound on branch 52, wounded May 1. 3. Same for wound on branch 69, wounded September 22.

some of the cysts are found slightly farther outwards from the remainder of the series, and so form a 'staggered' series. Occasionally, in addition to the first there are one or two later series which may be either in the summer wood of that year or in the early spring wood of the following year. Only in exceptional cases, however, are the later series of cysts extensive, and in order to clarify the nature of these exceptions they will be considered individually.

The large number of cysts recorded in Tables I and II for the wounds of branch 12 at ten and sixteen years and for the wound at nine years in branch 15 are due to accidental wounds of later date. The numbers of cysts in the first series at each of the wounds are 4, 1, and 7, whereas those recorded in Table I which include the later series are 30, 15, and 33.

The wound at twenty-four years on branch 72 is at the side of the branch opposite an unofficial wound of two years before. In the vicinity of the earlier wound there is one cyst in the year of wounding and none in

the following year, but in the third year, which is that of the experimental wounding, there are many. There are also numerous cysts at the experimental wound. It seems evident that the larger responses at both wounds are due to a cumulative affect.

There was a very extensive destruction of the cambium in the wound at twenty-one years in branch 72. It is possible that the greater number of cysts is related to the amount of tissue destroyed. The wounds at four years in stem 55 and at nine years in branch 17 have later series of cysts, but corresponding accidental wounds were not found. It may be noted from Tables I and II that throughout branch 17 there is a greater production of resin tissue than in the other branches wounded before the growing season.

In at least four of the seven wounds considered above there has been summation of the stimuli of the experimental and adjacent accidental wounds in connexion with the production of large responses. These four wounds, as well as the one with the large area of destruction, are marked by asterisks in Tables I and II. Their resin cysts are not included in the average figures given at the bottom of each table.

Resin tissue is very extensive about growing period wounds, cysts sometimes being found as much as 6 cm. above and 2 cm. below the wound centre. This extent is similar to that noted by Tschirch (8) in *Abies*. As may be seen in the lower row of Text-fig. 1, the resin tissue about each wound is extended in the vertical rather than the tangential direction. This may also be noted in Pl. XIX, Fig. 4, where there are only three cysts on one side of the wound, whereas at 1 cm. above (Pl. XIX, Fig. 3) the series is quite extensive, and even at 4 cm. above (Pl. XIX, Fig. 2) there are many cysts. The cysts form an anastomosing network of cavities which ramifies throughout practically the whole of the extensive parenchymatous tissue. This is in contrast to the responses of the dormant period wounds where the cysts occupy only a small part of the less extensive parenchymatous tissue.

Where the cysts are numerous, as in the June and July wounds, they occasionally open into the wound cavities. Where they are isolated and at some distance above or below, as in the responses to dormant period wounds, such openings are rarely seen.

Above and below growing period wounds there is an upward and outward, or downward and outward, extension of the parenchyma tissue. The resin cysts which appear in this parenchymatous mass above and below the wound are therefore a short distance outward from the inner limit of wound tissue. In a fairly vigorous branch or stem this radial distance may be as much as 0.2 mm. The series of resin cysts, so formed outwards from the wound, extends upwards or downwards without further radial displacement. Four or five centimetres above the wound the series is the

same distance out in the ring as just above the wound. Towards the upper limits, however, there are occasional cysts which just before they die out pass outwards a short distance from the remainder of the series. The greatest amount of such an outward displacement observed was 35  $\mu$ .

#### FACTORS OTHER THAN THE TIME OF WOUNDING.

In addition to the time of wounding there are other factors which may have varying effects upon the production of resin tissue. These are: vigour of growth, cambial age at the points of wounding, wound size, amount of tissue destruction, distances between wounds, and the type of wood in which the wounds are found.

Vigour of growth is regarded by Kirsch (4) as the causal factor in the development of resin canals in the pine. According to Jeffrey (3) resin cysts may appear in certain vigorous annual rings as a reversion to an ancestral character, for example, the resin cysts in the vigorous first year of the branch of *Sequoia gigantea*. In *Pinus sylvestris* Münch (5) noted a direct relation between the number of resin canals and the annual ring width. In *Tsuga canadensis* the writer (1) observed that traumatic resin cysts were most abundant in the vigorous specimens. On the other hand, Thomson and Sifton (7), in the case of *Picea canadensis*, failed to find a direct correlation between resin-canal production and vigour of growth, whether determined in terms of length or thickness.

In order to determine if the greater resin-cyst production of the specimens wounded in June and July was to be attributed to increased vigour of growth rather than to the time of wounding, the numbers of resin cysts and the vigour of the different stems and branches were compared. The vigour was considered only in terms of growth in the radial direction, that is in annual ring width. The average width of the wounded year throughout each branch and stem was determined from the average of four measurements near each wound, the maximum and minimum widths 2 cm. above and below (or 1 cm. where the 2 cm. section was not available).

The average ring width and number of resin cysts in the wounded branches are shown in Text-fig. 4. For branches 56-60 (the month of June in the figure) the curves representing ring width and resin-cyst production follow a similar course, but in the case of branches 63-7 (July) there is a decided divergence. The outstanding feature is the increase of resin cysts in the branches wounded in June and July. This is also shown in Table IV for the branches of two trees. The numbers of resin cysts are clearly correlated with the time of wounding, rather than with differences in ring width. The comparison also shows that the more numerous cysts of the branches wounded in July are not attributable to inherent differences in the trees.

A similar situation holds for the wounded stems. Stems 55 and 61 (Table V) are of approximately equal vigour, but 61, wounded in the growing season, has many more cysts than 55. A like comparison holds for 50 and 66, though here the stem wounded during the growing season is the less vigorous of the two.

TABLE IV.

*Wounded Branches of Trees A and B.*

Tree.	Branch.	Date of wounding.	Ring width in mm.	Maximum number of cysts per wound.
A.	10	March 11	0.50	3
	14	April 21	0.23	3
	1	July 17	0.32	58
B.	11	March 11	0.32	3
	2	July 17	0.28	56

(All wounds are  $\frac{1}{8}$  inch.)

TABLE V.

*Wounded Stems.*

Stem.	Date of wounding.	Ring width in mm.	Maximum number of cysts per wound.
50	April 13	0.8	6
55	May 12	1.8	10
61	June 30	1.9	64
66	August 12	0.5	36

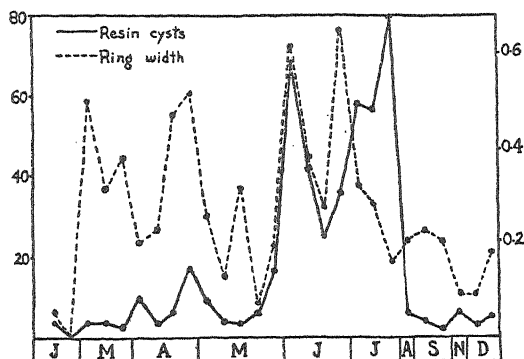
(Wounds on stems 50, 61, and 66 are  $\frac{1}{8}$  inch, and on stem 55  $\frac{3}{16}$  inch.)

It is evident that the greater number of resin cysts about the June and July wounds is not attributable to vigour of growth. On the whole the differences in the average ring width of the specimens wounded before and after the growing season, and those wounded during the growth period, are not great. Only in the branches wounded in January could vigour be regarded as a limiting factor.

Another factor which might possibly influence the production of resin tissue is that of cambial age. The experimental branches are of similar size and the wounds equidistantly spaced, but owing to the varying rates of growth some of the branches are of different ages at the points of wounding. In most branches, however, the wounds are within similar age limits, as shown by the fact that 85 per cent. of the growing period and 87 per cent. of the dormant period wounds are in the ninth to twenty-fifth years inclusive. Accordingly, the wounds of both the dormant and growing periods are subject to similar conditions in respect to cambial age. As the

experimental branches were located on trees of similar size, and probably of like age, the age of the trees could scarcely be considered a variant factor.

Wound size, however, varied; chisels of two sizes were used, the



TEXT-FIG. 4. Average resin-cyst production (data from Table I) and ring width of the wounded branches. Vertical axes represent number of cysts on left side, and ring width in mm. on the right. The month of wounding is given at the bottom (ten months of the year—no wounding in February or October).

wounds in branches 51, 56, 58, 3, 68, and 72 having been made by the  $\frac{3}{16}$ -inch chisel and the remainder with the  $\frac{1}{8}$ -inch chisel. In the wounds of the growing period, where the tangential series of cysts are as wide as the wounds, the wider wounds have the more extensive series. This is to be observed on comparison of branches 58 and 3 with the others. In wounds of the dormant period, where there are isolated cysts only, the differences in number of resin cysts between the  $\frac{1}{8}$ - and  $\frac{3}{16}$ -inch wounds are insignificant. The two wound sizes in no way nullify the conclusions regarding the relationship between seasonal wounding and resin-cyst production. It is readily seen that the  $\frac{3}{16}$ -inch wounds of the growing season have more cysts than the same sized wounds of the dormant period. This also applies to the  $\frac{1}{8}$ -inch wounds.

Since the wounds were not covered by any protective coating it might be expected that there would be variations in the amount of tissue destroyed, correlated with the season of wounding. The average maximum tangential destruction of the cambium was 4.0, 4.3, and 4.6 mm. for wounds inflicted prior to, during, and after the growth period (for wounds where the  $\frac{1}{8}$ -inch chisel was used). The differences are not marked, and consequently may not be significant. The vertical extent of the cambial destruction was usually 1.0 to 1.2 cm., but sometimes was greater; in branch 72 (Dec. 8) it varied from 1.6 to 3.0 cm., the largest wound having the greatest resin-cyst production (wound at twenty-one years, Tables I and II). In the case of one wound of branch 70 (Nov. 3) the vertical

extent was 1.6 cm. Only seven wounds showed a larger amount of tissue destruction, and of these only one a corresponding increase in resin cysts.

The distance between the wounds on all branches and stems is constant at 23 cm. and accordingly there could scarcely be a cumulation of stimuli or responses in some branches and not in others. Even in the growing-period wounds the maximum vertical extent of resin tissue is slightly over 8 cm., which is far short of the 23 cm. space between adjacent wounds. In all the branches the wounds were on the upper, soft or white, wood side, and so differences in the amount of resin tissue cannot be attributed to difference in wood type.

### SUMMARY.

In the branches experimentally wounded during June and July extensive series of resin cysts were produced in response to wounding. In contrast, the responses to wounds inflicted during the dormant period, August to May inclusive, were meagre. The growing season of the experimental material was practically limited to June and July. There is evidently a definite relationship between cambial activity and the production of resin tissue. Other possible factors, such as vigour of growth, &c., do not appear to have played an important part in the determination of the results.

My thanks are due to Professors R. B. Thomson and H. B. Sifton for the experimental material, and Professor Thomson for suggestions and criticisms during the course of the investigation.

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### EXPLANATION OF PLATE XIX.

Illustrating Mr. M. W. Bannan's paper on 'Seasonal Wounding and Resin-cyst Production in the Hemlock, *Tsuga canadensis* (L.) Carr.'

Fig. 1. Branch 53, wounded May 12, section 1 cm. above wound; parenchyma cells present in the earliest spring wood but resin cysts absent.

Fig. 2. Branch 58, June 15, section 4 cm. above wound; portion of series of resin cysts.

Fig. 3. Section 1 cm. above same wound; series of resin cysts.

Fig. 4. Section through the wound; three resin cysts at one side.

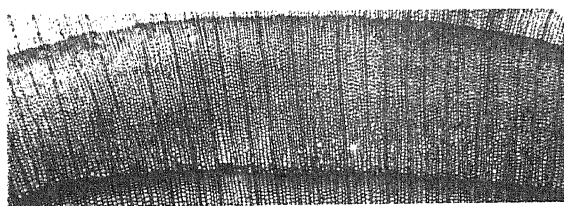
Fig. 5. Branch 3, July 17, 1 cm. above wound; part of series of resin cysts.

Fig. 6. Branch 68, Sept. 22, section 1 cm. above wound; parenchyma cells and resin cysts both absent.

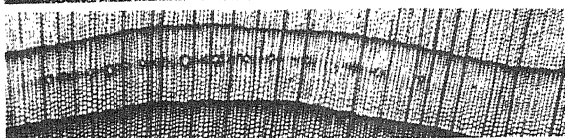
Fig. 7. Branch 73, Dec. 8, 1 cm. above wound; neither parenchyma cells nor cysts present.

Fig. 8. Section 1 cm. below same wound; parenchyma cells in the spring wood of the year following wounding.

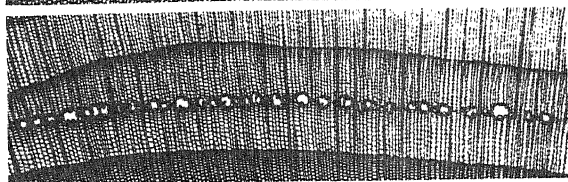




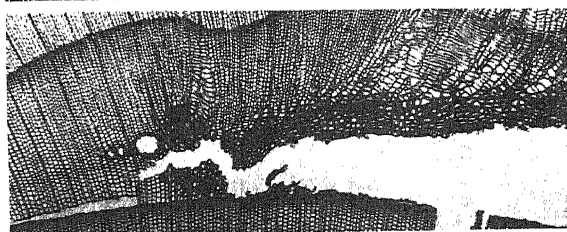
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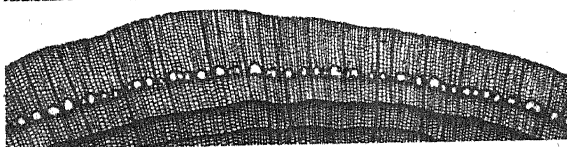
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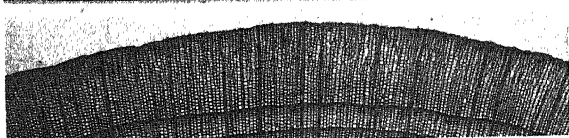
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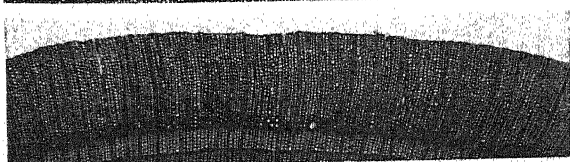
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# Some Observations on the Relation between the Hydrogen-ion Concentration of the Soil and Plant Distribution.

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## I.

THE relation between the hydrogen-ion concentration of the soil and the incidence of species in natural vegetation has been the subject of a large number of investigations (references 1 to 7). As an example may be cited the extensive observations made by Olsen (5) on meadows and woodlands in Denmark. In every locality which Olsen examined he measured the pH value of the soil, and noted the presence or absence of a number of species on ten trial areas of 0.1 square metres. The frequency of a species in a given locality was expressed as a percentage of these ten quadrats in which it occurred. The range of soil reaction, from pH 3.5 to pH 7.9, was divided into nine classes, 3.5-3.9, 4.0-4.4, 4.5-4.9, and so on. The average frequency of any species in each class was calculated by adding the frequencies from different localities, and dividing by the number of localities in which the species occurred. Olsen's work may be criticized on the grounds that the frequencies measured depend on the size of the quadrats used, and that the nine pH classes do not represent equal ranges of hydrogen-ion concentration, since the relation between hydrogen-ion concentration and pH is logarithmic. The results do, however, show what Olsen claims for them, namely, that for each species there is a characteristic range of pH outside which it does not occur. Similar conclusions have been reached by F. Chodat (4) in Switzerland, by Atkins (1, 2) in Britain, and by Christopherson (6) in Norway.

Several workers, notably Salisbury (7) in this country and Braun-Blanquet (3) on the Continent, have claimed to show the occurrence of one

or more pH optima within the range favourable to a species. Salisbury plotted against pH classes the number of localities in which various species were found, and obtained curves for *Pteridium aquilinum*, *Vaccinium myrtillus*, and *Scilla nutans*, which had the form of normal frequency curves. Curves constructed in the same way for *Mercurialis perennis* and *Ficaria verna* appeared to be bimodal, although no test of bimodality was applied. Salisbury concludes that the modes of these distributions represent pH optima for the species concerned. All these curves were obtained by measuring the pH wherever the species under investigation was found, no account being taken of the pH of soils on which the species did not occur. There is consequently no means of determining whether the curves are distribution curves of the species concerned in relation to pH classes, or merely distributions of the acidity of the soils themselves irrespective of the vegetation upon them.

In justification of his conclusions from these data Salisbury suggests that all distribution curves of soils would have their modes at the same pH value, whereas the curves he obtained had not. This would be true if all the samples had been taken at random, but Salisbury's method of sampling will inevitably produce soil distribution curves with different modes, because each curve represents a soil of different type. Thus, the curve for *Mercurialis* represents woodland soils, that for *Pteridium* heath and moorland soils, and that for *Scilla nutans* the soils of coppices and similar habitats.

It is clear that, before any conclusion can be drawn as to the influence of pH upon a species *within* its 'pH range', the data must be collected in such a way that the random distribution of soil acidity can be separated from the distribution of the species in question with respect to soil acidity. In the present paper a technique for collecting adequate data is outlined, and some preliminary results are given to illustrate its use.

## II.

To separate the frequency distribution of a species over an area from that of the soil acidity over the same area, it is necessary to sample the area at random, and irrespective of whether the species is growing on the sample plot or not. The distribution of the species with respect to pH may then be analysed by statistical methods. This has been done for two species, *P. aquilinum* and *V. myrtillus*, in four localities in Britain, and the technique may best be illustrated by a description of the actual experimental procedure.

### Experiment 1. Denbighshire.

The area selected for observation was a stretch of moorland between Ruthin and Mold. The object of the experiment was to determine

whether there is any relation between the incidence of *P. aquilinum* and *V. myrtillus* and the pH of the soil within the pH range of these species.

To ensure random sampling arbitrary transects were drawn on an Ordnance Survey map, representing a distance of about five miles. These transects were then followed by compass bearing, and at intervals of fifty yards along them samples of soil were collected at a depth of six inches, and the presence or absence of *Pteridium* or *Vaccinium* noted on a quadrat ten square feet in area.

The pH values of the soil samples were determined by Gillespie's 'Drop' method (8). Where there was any difference from replicate samples from the same plot the values were averaged; this was rarely necessary. The results are set out in Table I in the form of frequency distributions in classes of pH.<sup>1</sup>

TABLE I.

*Frequency Distributions of Occurrence of Pteridium and Vaccinium in Classes of pH.*

pH class means.	Number of samples.	Frequency of		Percentage occurrence :	
		<i>Pteridium</i> .	<i>Vaccinium</i> .	<i>Pteridium</i> .	<i>Vaccinium</i> .
4.8	2	0	2	—	100
4.9	2	0	2	—	100
5.0	2	0	2	—	100
5.1	5	1	4	20.00	80.00
5.2	13	6	8	46.15	61.54
5.3	17	8	11	47.07	64.71
5.4	7	4	3	57.14	42.86
5.5	44	27	28	61.36	63.64
5.6	78	41	54	52.56	69.23
5.7	16	5	12	31.25	75.00
5.8	7	5	1	71.43	14.29
5.9	9	4	3	44.44	33.33
6.0	7	5	1	71.43	14.29
6.1	3	2	0	66.67	—
		Mean percentages		51.772	62.992

It will be observed from the table that the distributions of *Pteridium* and *Vaccinium* have well-defined modes at pH 5.6, but that the distribution of the number of samples has a mode at the same class. It is necessary to derive from the data the distributions of the species irrespective of the number of samples taken at each pH. These distributions are given by the percentage of samples in each pH class in which the species occur. The values are set out in columns 5 and 6 of Table I; thus of the seventeen samples which fall into the 5.3 class *Pteridium* occurs in eight, i.e. in 47.07 per cent. It will be seen that the distributions of percentage occurrence are irregular, and appear to be bimodal.

<sup>1</sup> It is admitted that the pH is not a linear measure of the hydrogen-ion concentration, and that the readings should be classified in cH values. This is impracticable, however, for with increasing cH the errors on the corresponding pH values increase until the data become worthless.

The significance of the frequency distributions of species in pH classes may now be tested by *assuming* that the species occur with the same percentage frequency at every pH value within their range, i.e. are *independent* of pH within their 'pH range'. The theoretical distributions obtained on this assumption may then be compared with the observed distributions and the agreement tested by means of the  $\chi^2$  table given by Fisher (9). The theoretical distributions were calculated from the mean percentages given in Table I. It was assumed that 51.772 per cent. of the samples in any pH class would contain *Pteridium*, and 62.992 per cent. of the samples in any pH class would contain *Vaccinium*. In Tables II and III the observed and calculated distributions are compared.

TABLE II.

*Comparison of Observed Frequencies of Pteridium in pH Classes, with Calculated Values on the Assumption that Pteridium is present in 51.772 per cent. of the Samples at all pH Values.*

pH class means.	Frequencies of <i>Pteridium</i> observed.	calculated.	$\chi^2$ .
4.8	0	1.035	3.870
4.9	0	1.035	
5.0	0	1.035	
5.1	1	2.589	
5.2	6	6.730	0.079
5.3	8	8.801	0.073
5.4	4	3.624	0.039
5.5	27	22.780	0.782
5.6	41	40.382	0.010
5.7	5	8.284	1.302
5.8	5	3.624	0.479
5.9	4	4.659	
6.0	5	3.624	
6.1	2	1.553	
Total			6.634
			$n = 7$
			$P = 0.5$

It is clear from Tables II and III that the distributions of the two species within the range of pH investigated conform with the assumption that *within* their pH ranges the frequency of the species is not dependent upon the particular pH value of the soil. There is, in fact, no evidence of an optimum pH within the limits of the experimental data. The distributions with marked modes, given by the original data in Table I, columns 3 and 4, do not represent the effects of pH on the occurrence of the species but the distribution of acidity in random soil samples. It is of interest that the greatest contributions to the  $\chi^2$  values are those from the upper and lower pH classes.

TABLE III.

*Comparison of Observed Frequencies of Vaccinium in pH Classes, with Calculated Values on the Assumption that Vaccinium is present in 62.992 of the Samples at all pH Values.*

pH class means.	Frequencies of <i>Vaccinium</i> observed.	calculated.	$\chi^2$ .
4.8	2	1.259	1.362
4.9	2	1.259	
5.0	2	1.259	
5.1	4	3.150	
5.2	8	8.189	0.004
5.3	11	10.709	0.008
5.4	3	4.409	0.450
5.5	28	27.716	0.003
5.6	54	49.134	0.482
5.7	12	10.079	0.366
5.8	1	4.409	2.636
5.9	3	5.670	1.257
6.0	1	4.409	4.458
6.1	0	1.890	
Total			11.026
			$n = 9$
			$P = 0.25$

#### Experiment 2. Kent and Sussex.

The distribution of *Pteridium* in relation to pH was studied in three districts of the south of England, Ashdown Forest in Sussex, and Limpsfield and Keston Commons in Kent. The technique employed was precisely that outlined for Experiment 1 (p. 87c), except that the pH values were found by means of a quinhydrone electrode, and samples were taken every 100 ft. along the transects. In all 207 observations were taken. The data for the three localities are grouped together for analysis. The results are summarized in Table IV.

The frequency distribution of *Pteridium* in these localities appears on inspection to be bimodal. The principal mode is at pH 5.2, whereas the mode obtained for the distribution of *Pteridium* in Denbighshire is at pH 5.6. These differences are obviously due to differences in the mean soil acidity in the two regions. The calculated values in column 4 were obtained on the assumption that the percentage frequency of *Pteridium* is constant at all pH values within the pH range of the species. The average percentage frequency is 48.716; e.g. in the column of calculated values 0.487 is 48.716 per cent. of 1; 9.743 is 48.716 per cent. of 20, and so on. From the total  $\chi^2$  value it is clear that the distribution of *Pteridium* in the three localities in Southern England does not differ significantly from the assumed distribution; in other words, pH has no effect on the distribution of *Pteridium* between the values pH 4.7 and pH 6.2. It may be

noticed again that the greatest contributions to the value of  $\chi^2$  come from the extreme classes of the distribution.

TABLE IV.

*Frequency Distribution of Occurrence of Pteridium in Classes of pH.  
Kent and Sussex Data.*

pH class means.	No. of samples.	Frequency of <i>Pteridium</i> observed.	Frequency of <i>Pteridium</i> calculated.	$\chi^2$ .
4.7	1	0	0.487	0.018
4.8	5	2	2.436	
4.9	7	4	3.410	
5.0	20	5	9.743	2.309
5.1	32	14	15.589	0.162
5.2	45	20	21.922	0.169
5.3	20	10	9.743	0.007
5.4	20	8	9.743	0.312
5.5	8	3	3.897	0.878
5.6	8	4	3.897	
5.7	10	9	4.871	
5.8	12	7	5.846	0.227
5.9	10	4	4.871	2.948
6.0	7	4	3.410	
6.1	1	0	0.487	
6.2	1	0	0.487	
Total				7.066
				$n = 8$
				$P = 0.6$

Finally, it is instructive to pool the data from 212 observations in Denbighshire and 207 observations in Southern England, and to test the assumption of independence on the combined data. The analysis is given in Table V. The value of  $P$  is about 0.82, which is conclusive evidence that the distribution of *Pteridium* is determined by factors other than pH, notwithstanding the apparent modal distribution of the species in pH classes.

It is obviously of importance to discover how the soil acidity affects the distribution of vegetation. It is established beyond doubt that many species can grow only within a definite pH range, but there is at present no evidence from field observations that *within* the range of tolerance the frequency of any species is influenced by soil acidity. It is clear from the foregoing examples that the distribution of *Pteridium* and *Vaccinium* between pH 4.7 and 6.2 is *independent* of pH, and is determined by other factors. In water-culture experiments a modal frequency distribution of growth rate in pH classes has often been observed, but it is not surprising that the results of such simple experiments are not applicable to the distribution of vegetation under field conditions.

If the soil acidity does not influence the incidence of species within their pH range, soil acidity cannot be invoked to explain the vagaries of



plant distribution within an association. By the application of the technique described in this paper it will be possible to establish the degree of significance of pH and other environmental factors on the distribution of species.

TABLE V.

pH class means.	No. of samples.	Frequency observed.	Frequency of <i>Pteridium</i> calculated. <sup>1</sup>	$\chi^2$ .
4.7	1	0	0.468	0.483
4.8	7	2	3.278	
4.9	9	4	4.215	
5.0	22	5	10.303	2.730
5.1	37	15	17.327	0.313
5.2	58	26	27.162	0.050
5.3	37	18	17.327	0.026
5.4	27	12	12.644	0.033
5.5	52	30	24.352	1.310
5.6	86	45	40.275	0.554
5.7	26	14	12.176	0.273
5.8	19	12	8.898	1.081
5.9	19	8	8.898	0.091
6.0	14	9	6.556	0.911
6.1	4	2	1.873	0.050
6.2	1	0	0.468	

Total 7.905

 $n = 12$  $P = \text{circa } 0.82$ 

## SUMMARY.

In studying the influence upon plant distribution of hydrogen-ion concentration in the soil, it is necessary to collect the data in such a way that the frequency distribution of soil acidity can be separated from the frequency distribution of the species in relation to soil acidity. In the present paper there is described a suitable technique for the collection of such data.

An analysis of the data from four localities shows that between pH 4.7 and pH 6.2 the incidence of *Pteridium aquilinum* and *Vaccinium myrtillus* is not determined in any way by the pH value of the soil; in other words, there is an equal chance of finding these species at any soil pH with the range of values investigated.

The ecological significance of this independence of soil pH and the distribution of species is briefly discussed.

<sup>1</sup> On the assumption that an average of 46.831 per cent. of all samples contain *Pteridium*.

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# Studies in Growth Analysis of the Cotton Plant under Irrigation in the Sudan.

## I. The Effects of Different Combinations of Nitrogen Applications and Water-supply.

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With twelve Figures in the Text.

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### INTRODUCTION.

THE study of the relationship of the growing crop to its environment may be regarded as a test of the claim that plant physiology has more than purely academic interest. There are two alternative methods of

approach. By the first, adopted mainly by agriculturists, attempts are made to relate the crop yield to the amount of one or more of the external factors by some 'Law of Limiting Factors' (Leibig) or by an expression of the 'Law of Diminishing Returns' (as e.g. in Mitscherlich's 'Wirkungsgesetz der Wachstumsfaktoren' or Maskell's 'Resistance Formula'). The present position of such treatments is outlined in E. J. Russell's 'Soil Conditions and Plant Growth'. The results of an experiment on the interrelation of the factors controlling the yield of cotton under irrigation in the Sudan, in which the present author was concerned, have already been published (8). In that paper emphasis was laid on the necessity of considering simultaneous action of diverse factors, and the end in view was to establish what were the optimal relations between the various factors.

In the second method the morphological and physiological processes resulting in the final crop are studied and the measurements deal with rates of change and growth curves rather than with the integration of these values as expressed by the final crop yield. This type of study originated in the classical work of W. L. Balls (2) on the analysis of the yield of the cotton plant in Egypt. Balls dealt primarily with flower curves and his range of observations was too limited for a complete analysis of crop production. The method was developed by Gregory and his collaborators (5, 6, 7, 8, 9), by Maskell (10), and by Briggs, Kidd, and West (3).

The above distinction between the two aspects is convenient for the presentation of data and for discussion. In the present paper emphasis will be on developmental studies and their relationships to the yields of cotton.

#### DESCRIPTION OF EXPERIMENT.

The experiment was carried out on cotton grown under irrigation at the Gezira Research Farm, Wad Medani, Sudan, during the season 1928-9. The factors studied were variations in the water supply ('water duty') and in the nitrogen supply. Details of the treatments and cultivations were as follows:

Total area:  $4\frac{1}{2}$  feddans (each plot was  $1/12$  feddan).<sup>1</sup>

Date of sowing: August 20th, 1928.

Sulphate of ammonia: applied October 6th, 1928.

Spacing: Two plants per hole, 50 cm. between holes along ridges, and 80 cm. between ridges.

Plants thinned: October 1st.

Irrigation: every fourteen days after mid-September.

<sup>1</sup> 1 feddan = 1.038 acres. 1 rotl = 0.99 lbs.

*Experimental Treatments.*

*Water duties* (from early October onwards).

L. Light (200–250 cubic metres per feddan).

M. Medium (350–400 " " " " ).

H. Heavy (500–550 " " " " ).

*Nitrogen applications.*

1 Nil.

2 Single dressing (300 rotls<sup>1</sup> ammonium sulphate per feddan).

3 Double dressing (600 rotls " " " " ).

Each nitrogen application was given for each water duty making nine treatments. Combinations of the letters L, M, H, with the figures 1, 2, 3, are used throughout the diagrams to denote the treatments. Each treatment had sixfold replication, giving fifty-four plots in all.

The land occupied by the experiment was under cotton and artificial irrigation for the first time, but it had previously been cropped with rain-grown dura. The seed used was Sakellaridis variety, main farm crop (Tokar origin). The spacing of the crop and the dates of sowing, thinning, and ammonium sulphate application were chosen as being the most likely, in view of previous results at the Gezira Research Farm, to be optimal. Owing to the risk of flooding, and the difficulty of ensuring that adjacent plots received their allotted water duty, the layout was arranged so that six plots on any one water channel were of the same water duty. The distribution of the water duties among the nine channels, and of the six nitrogen treatments within any one channel, was at random. Since the measurements on the plants necessitated walking on the irrigated soil, duck-boards were used to obviate mechanical injury to the roots and puddling of the soil by treading on the wet soil after an irrigation. The total amount of water given to the whole experiment at each irrigation varied during the season with the dryness of the soil, but, on all occasions after early October, there were similar differences between the light, medium, and heavy waterings. As young cotton plants are very susceptible to damage from heavy irrigation, differential irrigations were not introduced until October, in order to obtain a uniform stand.

Damage from blackarm (*B. malvacearum*) was negligible, but thrips caused light damage to the northern part of the experiment during late October: further damage was avoided by spraying. Leaf curl commenced during October and was widespread on all treatments before the end of the experiment.

<sup>1</sup> 1 kantar = 315 rotls.

## DEVELOPMENTAL OBSERVATIONS.

(a) *Heights, node numbers, and internodes.*

The normal cotton plant consists of a main stem bearing monopodial branches of unlimited growth in the axils of the leaves at the base of the plant. Higher on the main stem sympodia are produced in the axils of the leaves and bear at each node a terminal flower, growth being continued by a lateral bud. The sympodia may thus have a considerable number of flowers of varying morphological order. The whole morphology of the plant has been well described by Balls (2).

The plants used in this experiment are characterized by limited monopodial and sympodial growth. In consequence, the successive phases of leaf, flower, and boll production are clearly defined in time, which facilitates observation.

The observational data were chosen to reflect the results of different physiological activities. The meristematic activity is reflected by the number of nodes produced on the main stem and by the number of flower-buds on the sympodia, from which the rate of development of new parts may be determined. The growth of the individual parts of the main stem is measured by the internode length, derived from node numbers and heights. The potential cropping power is determined by flower counts, and the flower productivity by boll numbers. As the cotton flower remains open only for a few hours, the separate daily totals represent the actual rates of flower production. Limitation of the crop by shedding of developing bolls is derived from the series of flower and boll counts.

For observation purposes the plots were subdivided, half of each being reserved for final yields and for selected observation plants, and half for the removal of plants at the whole-plant sampling.

The frequency and number of observations are shown below :

Observation.	Frequency of observation.	Per plot.	Per treatment.	Total.
Node numbers	14 days	5 pairs	30 pairs	270 pairs
Heights	14 days	10 "	60 "	540 "
Flower numbers	Daily	15 "	90 "	810 "
Boll numbers	Daily	15 "	90 "	810 "

Immediately after thinning, plants for observation were selected at random within the limits imposed by the conditions: (1) correct spacing, (2) plants per hole, and (3) within the four central rows of each plot, for convenience when using duckboards. These restrictions still allowed a random choice of one hole in eight, at least.

The results of the observations are presented below in a uniform manner. Each table shows the average values of the six replicates of each of the nine treatments. Each row or column represents the effect of

increase of water or nitrogen respectively, while the other factor is maintained at a constant level. The marginal totals represent the effects of water and nitrogen averaged for the three levels of the other factor. The general mean is also included. The appropriate standard error (S.E.) for comparisons of treatment effects is given with each Table. (Those for 'water' and 'nitrogen' relate to the appropriate marginal means, and those for 'interaction' to the individual values within the set of nine.)

The average heights of the main stem and the average numbers of nodes per plant on the main stem are given in Table I for two dates of observation. The average internode lengths of the main stem at the end of the experiment are also included.

At both dates the marginal totals show increases in both height and node numbers, with increasing nitrogen and water supply. The effect of nitrogen is considerable and increases with advancing age, but the effect of water is much less marked. Examining in turn the rows entered in the table, it is seen that the extra height obtained for an increment of water increases with each addition of nitrogen. Similarly from the columns, the rate of increase of height with nitrogen supply also increases with the amount of water. These variations in the action of one factor at the different levels of the other factor constitute the interaction of the two factors. On examining the node numbers in the same way, it is seen that these do not show regular differences at the first date, while at the second date each factor gives similar increases irrespective of the level of the other. Apparently, therefore, the effects of water and nitrogen interact in height development but not in node number. These conclusions are confirmed statistically.

Internode length shows a significant average effect of water supply, increase of water supply resulting in longer internodes. The average effect of nitrogen is very small, as may be seen from the marginal totals. There is, however, a large interaction effect, since with light watering increase of nitrogen decreases the internode length, whereas with heavy watering increase of nitrogen increases internode length. This interaction is confirmed statistically.

(b) *Length of main stem.*

The effects shown in Table I indicate clearly the different functions of water and nitrogen in determining the growth of the main stem. Node numbers show the effect of nitrogen directly. Meristematic activity is dependent on a ready supply of nitrogen and ceases when the level of nitrogen in the plant has fallen below a critical level. The time at which this level is reached is delayed with higher nitrogen supply, and hence node production continues for a longer period. In addition, the rate of production of nodes is limited, after the very early stages, by the nitrogen concentration within the plant, and, for this reason also, the higher node

TABLE I

*Heights, Node numbers, and Internode Lengths of Main Stem.*

Heights (cm.) (Nov. 26th).	{Water Nitrogen Interaction			S. E.	Node numbers (Nov. 26th).			{Water Nitrogen Interaction		
	Water.	L.	M.	H.	Water.	L.	M.	H.	S. E.	Mean.
Nitrogen	1	57.7	63.5	64.0	1	20.5	21.0	21.8	0.91	21.1
	2	63.9	68.6	73.4	2	22.9	22.7	20.7	0.24	23.2
	3	66.4	72.3	81.6	3	24.0	23.7	25.7	0.31	24.5
	Mean	62.7	68.1	70.0	Mean	22.5	22.5	23.9		22.9
Final heights (cm.).										
Nitrogen	{Water Nitrogen Interaction			S. E.	Final node numbers.			{Water Nitrogen Interaction		
	Water.	L.	M.		Water.	L.	M.	H.	S. E.	Mean.
	1	63.6	71.6	70.3	1	25.3	26.9	27.7	0.21	26.0
	2	71.1	78.8	83.2	2	30.0	31.1	31.8	0.5	31.0
Average final internode lengths (cm.).	3	78.1	84.8	95.1	3	34.7	35.4	36.1		35.4
	Mean	70.9	78.4	82.9	Mean	30.0	31.1	31.9		31.0
	{Water Nitrogen Interaction			S. E.	Average final internode lengths (cm.).			{Water Nitrogen Interaction		
	Water.	L.	M.		Water.	L.	M.	H.	S. E.	Mean.
	1	2.52	2.67	2.53	1	2.57	2.57	2.57	0.13	2.57
	2	2.38	2.53	2.63	2	2.51	2.51	2.51	0.05	2.51
Average final internode lengths (cm.).	3	2.25	2.40	2.65	3	2.43	2.43	2.43	0.07	2.43
	Mean	2.38	2.53	2.60	Mean	2.50	2.50	2.50		2.50



number is found with higher nitrogen supply. Water, on the other hand, has little effect on node production; apparently it is never so restricted as to prevent the meristematic activity. The height of the plant is determined by the product of node number and internode length. It has been seen that the effect of water on internode length is considerable and is highly significant. The effect of nitrogen on this character depends on the level of water. The reduction of internode length with increasing nitrogen, with light watering, is doubtless related to the greater number of nodes produced at the higher nitrogen level. Water supply is sufficient to allow meristematic activity to proceed, but it is not sufficient for the extension growth of the internodes thus formed, and therefore the individual internodes remain shorter the larger the number present on the plant. With increasing water supply this effect disappears, and a small increase in length of internode is obtained by increasing the nitrogen supply. There can be no doubt that the cell number is determined by the nitrogen supply and the size of the individual cells by the water supply. Both factors cause significant increases in heights. At all levels of water, the height is increased by the addition of nitrogen owing to the fact that, even at low water level, the increase in node number more than compensates for the decrease in internode length; whereas at high water level the two effects intensify each other.

The independence of water and nitrogen on meristematic and extension growth is of great importance in understanding the adaptations of the plant to periods of intermittent drought, such as occur under irrigation in the Sudan. In the periods between irrigations the water supply apparently never falls so low as to prevent the laying down of new parts, and meristematic activity, as measured by flower production, shows no periodicity corresponding with the periods of irrigation. When water supply is again increased, rapid extension growth may proceed so that the effect of fluctuations in water supply disappear.

*(c) Flower production.*

The curve of flower numbers, obtained from the daily flower counts and expressed as the total number of flowers produced up to a certain date, is of the usual sigmoid type, and has the same form for all treatments, although the slopes and final values vary with the treatments. This curve closely resembles in form the curve of an autocatalytic reaction as has been shown by Prescott (13).

The data for flower production are presented graphically in Fig. 1, which gives values for five-day overlapping means, derived from the daily totals for each treatment. The curves represent the deviations of the daily values of the separate treatments from the mean daily value of all treatments, over a period from November 12th to December 14th, which covers

the main flowering period. A point above the zero line in the diagram represents an excess of flower production over the mean and a point below a deficit. It is seen that the curves fall into three well-marked groups.

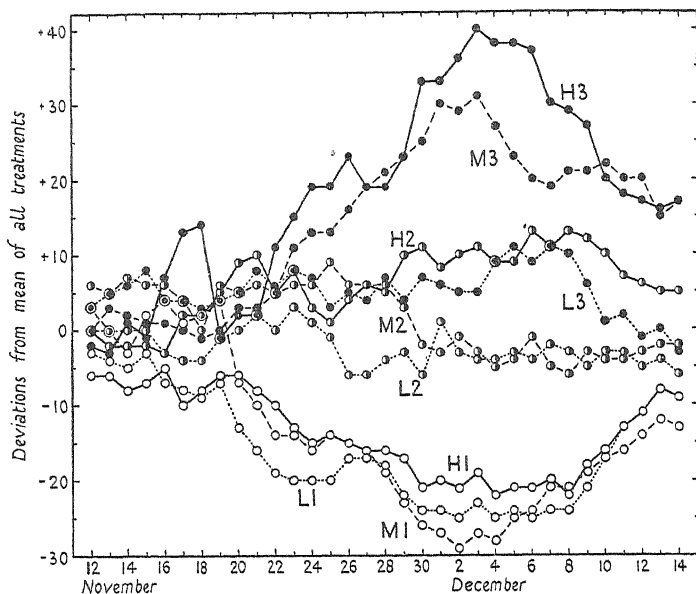


FIG. 1. Graph showing daily rates of flower production of separate treatments, as deviations from mean flowering rate of all treatments.

The lowest group contains all the plants, which receive no nitrogen dressing; in this group differences due to the differential waterings are small, but in general the heaviest water gives the highest values. The second group includes all the plants with the single dressing of nitrogen. For these, differences due to water are more marked, the heaviest watering (H<sub>2</sub>) lying above the zero line and the other two water levels (M<sub>2</sub>, L<sub>2</sub>) below it. This group also includes the treatment with a double nitrogen dressing and a light watering (L<sub>3</sub>), which gives a flower production almost equal to that produced by a single nitrogen dressing and heavy watering (H<sub>2</sub>). Further increase of water supply with the double nitrogen dressing is reflected in a large increase in flower production, as is seen in the third group. The interaction of water and nitrogen is thus clearly shown by this diagram. The increase in flower production due to increased water supply becomes progressively greater as the nitrogen level is raised. The type of interaction is therefore similar to, but much more pronounced than, that already seen for heights of plants. The diagram brings out a further point. At the commencement of flowering the nitrogen effects are not clearly marked, but, as flowering proceeds, the curves separate out according to differences of treatment. In the early stages of growth the number

of flower-buds laid down is limited by the development of the stem, for only a few positions are available for the production of flowers in the small number of sympodia then present. At this stage, as will be shown later, the level of nitrogen in the plant is high for all treatments. Nitrogen supply is therefore adequate to develop flowers in all available morphological positions irrespective of treatment. As the plant ages the number of available positions increases exponentially and the demand for nitrogen becomes increasingly great. The internal level of nitrogen falls as the demand overtakes the supply, and this is reflected in the falling rate of flower production in the low nitrogen series, as compared with those receiving nitrogen dressings. Eventually, in all series, internal starvation of nitrogen checks flower production so that the curves converge to the same low values.

For each nitrogen level increased water supply shows some increase in flower production. This effect of water may result from the sympodial arrangement of the flower buds. Although the flower bud is undoubtedly laid down early in the development of the sympodium it does not develop fully until the extension of the sympodium has occurred and the vascular supply differentiated. Water is needed for this extension growth, and, if the supply is inadequate, the potential formation of the flower is prevented.

The effects discussed above are shown clearly in the total flowers produced up to two dates (December 31st and April 30th), given in Table II.

The average effect of nitrogen, as seen from the marginal totals, is clear at both dates. The effect of water is much less marked, and, indeed, at the first date fails to reach statistical significance. The interaction effects are of the type already discussed and are found to be highly significant at both dates.

(d) *Boll production.*

The mature bolls produced on the plants observed for flower production were counted daily and separated into healthy and diseased bolls. The effect of the treatments on boll production may be assessed from the data presented in Table III for two dates.

At both dates the effect of nitrogen is marked and highly significant. It will be seen, however, that at the first date there is a *reduction* in number of bolls with increasing nitrogen supply, whereas at the later date there is a very large *increase*, of approximately 88 per cent. The effect of water supply on boll production is similar to that of nitrogen, i.e. a reduction of bolls at the early date followed by an increase. The effect of water on total bolls until December 31st does not attain statistical significance. At the later date a significant effect is obtained, but of a lower order than that of nitrogen. The decrease in boll production with increase of nitrogen

supply, early in the life of the plant, shows the well-known effect of delayed maturation due to excess nitrogen.

Examination of the data shows that variation in boll number with water supply is uniform at all levels of nitrogen up to December 31st and vice versa. There is, therefore, no interaction between the factors at this stage. At the second date there is a marked interaction similar to that with flower numbers and height measurements.

The boll numbers presented above include diseased bolls which make no contribution to yield. Similar effects are, however, obtained when healthy bolls alone are considered. A comparison of the tables for April 30th shows that the incidence of disease is small and is not affected by the level of nitrogen.

(c) *Boll shedding.*

The number of bolls shed during development are calculated from the flower and boll numbers and are given in Table III as a percentage of the total flowers produced. Least shedding occurs at the medium levels of both factors, but only the effect of water supply is significant. It has not been possible to explain these observations. There is little evidence here that water is the main factor in boll shedding, as was suggested by Balls (2).

#### SAMPLING DATA.

To determine the increase in weight of the cotton plants, the distribution of nitrogen within the plants, and the rate of removal of nitrogen from the soil, the whole plant sampling method developed by Gregory (5, 6) was used.

The whole plant samples were taken at 14-day intervals throughout the season (October until April), immediately preceding each irrigation. As root excavations on a field scale were impossible, sampling was restricted to the parts of the plants above ground level. At each sample five pairs of plants were removed from each of the special half-plots, the choice of plants being random except for the restriction that the pairs removed were correctly spaced. Thus 540 plants were removed on each sampling day and 8,100 plants during the course of the experiment.

For convenience the plants were separated into (1) 'green leaves', which represent the assimilating material, (2) 'stems', including also petioles, flower branches, and dried boll cases, which represent conductive and mechanical tissue, (3) 'flower-buds', including the open flowers, when present, and (4) 'green bolls', which represent the potential crop. The seed-cotton was picked separately, and later included in the total plant weights. The dead leaves and shed branches, buds, and bolls could not be collected satisfactorily, and were omitted from the sampling weights. This resulted in a decrease in total dry weight and total nitrogen content during December.

TABLE II.

Total flowers, produced until April 30th, per plant.						
S. E.		<div> <div>Water</div> <div> <div>2.94</div> <div>1.03</div> <div>1.28</div> </div> </div>			Mean.	
		Water.	L.	M.		H.
Nitrogen	1	11.23	13.47	12.84	12.52	
	2	14.51	17.56	19.13	17.57	
	3	18.69	24.93	26.74	23.46	
	Mean	15.18	18.79	19.57	17.85	

Total flowers, produced until April 30th, per plant.

Water.	S. E.			Mean.
	L.	M.	H.	
Nitrogen	1	11.23	13.47	12.84
	2	14.51	17.56	19.13
	3	18.69	24.93	26.74
	Mean	15.18	18.79	19.57
			Water { Nitrogen { Interaction	2.94 1.03 1.28

TABLE III.

Total bolls per plant, until Dec. 31st.

S. E.	{			Mean.
	Water	Nitrogen	Interaction	
Water.	L.	M.	H.	
Nitrogen	1	1.08	1.07	0.73
	2	0.87	0.53	0.44
	3	0.73	0.35	0.34
	Mean	0.89	0.65	0.51

Total bolls until April 30th (healthy and diseased).

Water.	L.	{		Mean.
		S. E.	(Water Nitrogen Interaction	
		0.49	0.65	
Nitrogen	1	6.80	8.77	7.76
	2	9.46	12.36	11.44
	3	11.23	15.64	14.59
Mean		9.16	12.26	11.27

Total healthy bolls per plant, until April 30th.

S. E.	{		S. E.	Mean.
	{			
Water.	L.	M.	H.	Mean.
Nitrogen	1	6.15	8.18	7.11
	2	9.03	11.34	11.44
	3	10.66	14.19	14.78
	Mean	8.62	11.24	11.11

Boll shedding as percentage of total flowers.

Water.	S. E.			{			Mean.
	L.	M.	H.	Water	Nitrogen	Interaction	
Nitrogen	1	38.32	34.68	39.83	1.76		37.61
	2	39.32	30.51	34.54	1.36		34.79
	3	39.67	35.52	36.34		2.25	37.18
	Mean	39.10	33.57	36.90			36.53

Dry weights were obtained for the groupings given above, and the material was ground in a disintegrator for nitrogen analysis. Lint was analysed separately, and in the ripening bolls the lint was removed from

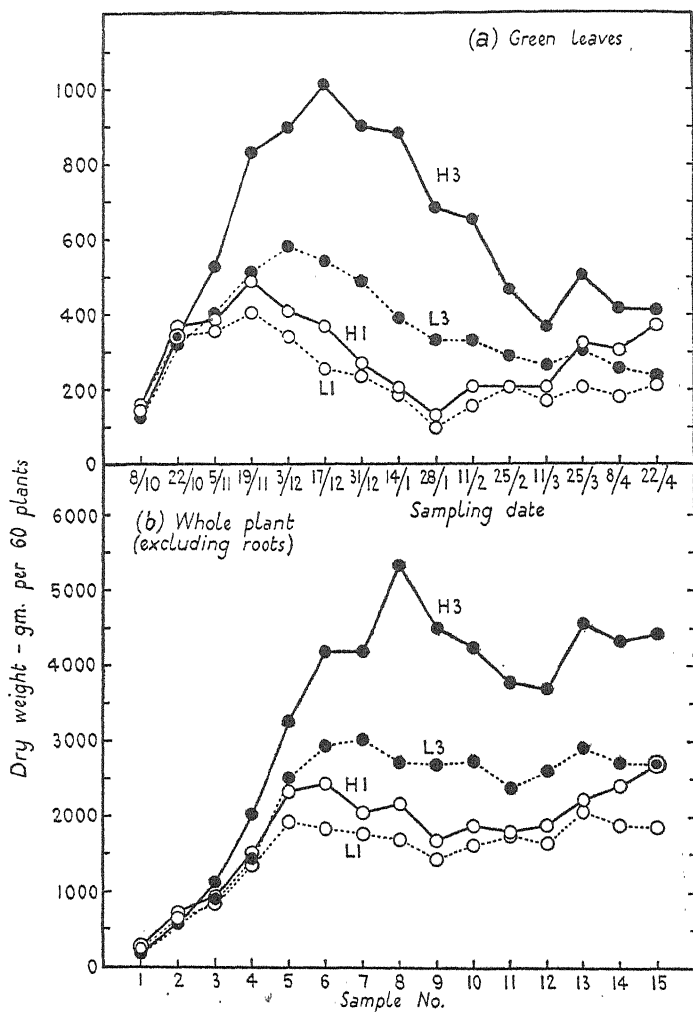


FIG. 2. Graphs showing dry weights of (a) green leaves and (b) whole plant for successive fortnightly samples, for the four extremes of treatment.

the bolls before grinding was possible. The nitrogen analyses for each sample were carried out in the Government Chemist's Laboratory, W.T.R. Laboratories, Khartoum.<sup>1</sup>

As root weights were omitted, the 'whole plant' weights include only

<sup>1</sup> The author wishes to record his indebtedness to Dr. Joseph and Mr. Whitfield, without whose extensive co-operation the samples could not have been analysed.

the aerial parts of the plants. The effect of the omission of root weights was small, for isolation of single plants showed that the dry weight of the whole root was small compared with that of the rest of the plant.

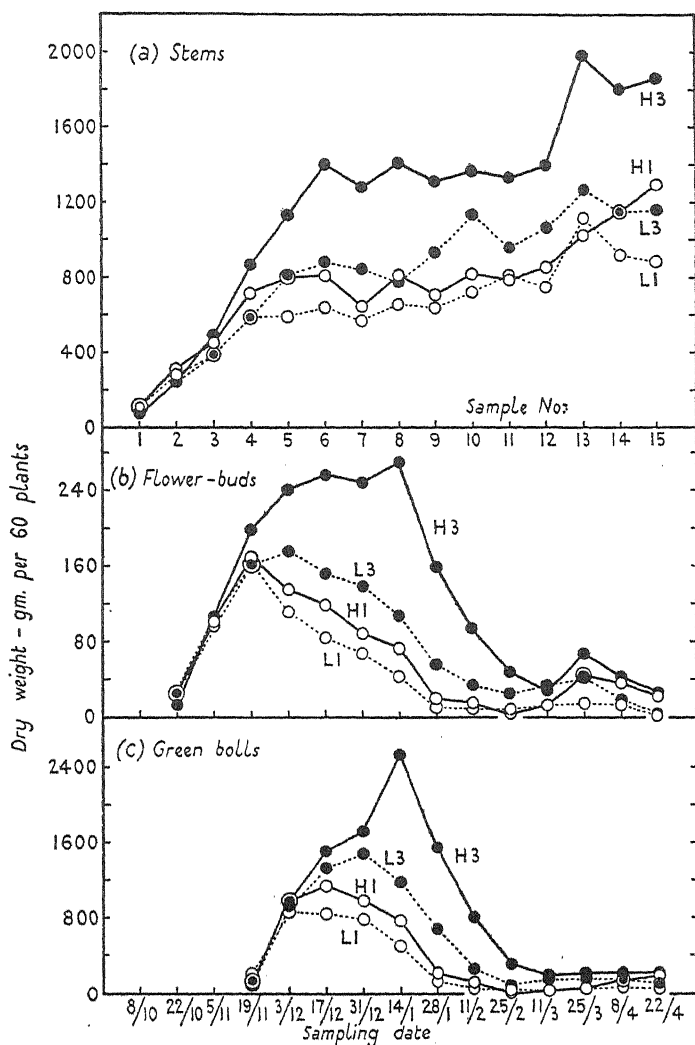


FIG. 3. Graphs showing dry weights of (a) stems, (b) flower-buds, (c) green bolls, for successive fortnightly samples, for the four extremes of treatment.

#### (a) *Dry weights.*

The dry-weight data of green leaves, stem, flower-buds, green bolls and of the whole plant, obtained fortnightly throughout the season, are summarized graphically in Figs. 2 and 3 for the four extreme treatments.

These curves show no differences in weight due to treatment at the time of the first sample, but with advancing age the curves separate. Those for no nitrogen application reach an early maximum and then decline, except for the stems. Those for plants receiving nitrogen applications continue to rise, and reach later and higher maxima. The effect of water supply is clearly shown, but, although the light watering gives low weights, the times of the leaf-weight maxima in the two series show no marked difference. The curves of green leaf weights reach a minimum value and then subsequently rise. The minimum occurs later in the series receiving the double nitrogen dressing, and again the position of this minimum is unaffected by the water supply. The secondary rise in the curves is due to resumption of growth after the first flush of bolls has matured. The earlier minimum in the low nitrogen series is due partly to earlier maturation, but mainly to the fact that the period of boll production is curtailed in these plants. The influence of the reproductive phase in limiting vegetative growth, shown experimentally by Eaton (4), is clearly demonstrated by these data. Similar effects are seen in the curves of flower-bud and green boll weights. The recovery in these cases is much delayed, since vegetative growth must precede flower production.

Statistical examination of the data shows that the nitrogen effect becomes significant at sample 3 (November 5th), and the water effect at sample 4 (November 19th). The interaction effects are very marked; the curves for low nitrogen diverge but little with water treatment, while with high nitrogen there are large differences due to water supply. This is seen in all the curves. The interactions are found to attain statistical significance at the time of sample 4 (November 19th).

(b) *Derived data.*

From the sampling data of dry weights, estimates of relative leaf growth rate, relative rate of total dry-weight increase ('efficiency index') and net assimilation rate were obtained by methods used by Gregory (6). (The derived data refer only to the growth of the aerial parts.) The net assimilation rate is calculated on the basis of unit weight of leaf instead of unit area, owing to the difficulty of estimating leaf area in the field.

The relevant data are entered in Table IV. Statistical examination showed that the interaction effects of these data were in no case significant: average values were therefore obtained by grouping the data under the headings of (a) increasing water and (b) increasing nitrogen. *Relative leaf growth rate* shows high values in the period between the first two samples (October 8th to 21st). After this they fall rapidly, attaining negative values (falling leaf weights) at the time of the fourth to fifth sample (November 19th to December 2nd). Statistical examination shows



that the effect of water on relative leaf growth rate is not significant, whereas the nitrogen effect is very highly significant.

TABLE IV.

*Growth Rates : Effects of Water and Nitrogen Supply.*

Sampling periods.	Date.	Relative leaf growth rate.					
		Water.			Nitrogen.		
		L.	M.	H.	1.	2.	3.
1-2	Oct. 8-21	5.10	4.94	5.42	4.92	4.70	5.83
2-3	" 22-Nov. 4	0.89	1.62	1.70	0.70	1.69	1.82
3-4	Nov. 5-18	1.24	0.86	1.99	0.65	1.40	2.04
4-5	" 19-Dec. 2	-0.13	0.76	-0.26	-0.40	0.07	0.69
5-6	Dec. 3-16	-1.04	-0.97	0.21	-1.56	-0.37	0.13

Sampling periods.	Date.	Relative rate total dry-weight increase (‘Efficiency Index’).					
		Water.			Nitrogen.		
		L.	M.	H.	1.	2.	3.
1-2	Oct. 8-21	5.41	5.50	5.84	5.45	5.16	6.13
2-3	" 22-Nov. 4	2.28	2.84	2.93	2.14	2.92	2.97
3-4	Nov. 5-18	2.78	2.44	3.07	2.39	2.71	3.19
4-5	" 19-Dec. 2	2.74	3.02	2.52	2.68	2.56	3.05
5-6	Dec. 3-16	0.69	1.09	1.06	0.21	1.19	1.35

Sampling periods.	Date.	Net assimilation rate.					
		Water.			Nitrogen		
		L.	M.	H.	1.	2.	3.
1-2	Oct. 8-21	9.66	9.90	10.44	9.98	9.24	10.79
2-3	" 22-Nov. 4	4.69	5.91	5.99	4.58	6.06	5.94
3-4	Nov. 5-18	7.36	6.36	7.60	6.75	6.90	7.67
4-5	" 19-Dec. 2	10.10	11.19	9.16	11.29	9.20	9.95
5-6	Dec. 3-16	3.43	4.69	5.78	1.01	7.09	5.80

*Relative rate of total dry-weight increase* (efficiency index) also shows a high value over the first period, after which it remains nearly constant until early December. Again the effect of water is found to be insignificant, whereas nitrogen shows a significant effect, which is of a lower order than the effect on relative leaf growth rate.

*Net assimilation rate* values show considerable fluctuations, no doubt associated with the variations in climatic conditions. Thus the highest actual assimilation rate is found in the period from sampling four to five in the treatment receiving no nitrogen application. That the fluctuations are in fact due to external factors is shown by the high correlations for the values for different treatments over corresponding periods. Only in the last period does there appear to be a real fall in assimilation rate, and this is the time when maximum leaf weight is passed. Statistical examination shows no significant effect of variation in either water or nitrogen supply.

Reviewing the data presented in this section, it is clear that nitrogen

has a predominant effect on relative leaf growth rate and a much smaller effect on efficiency index. Net assimilation rate is unaffected by either

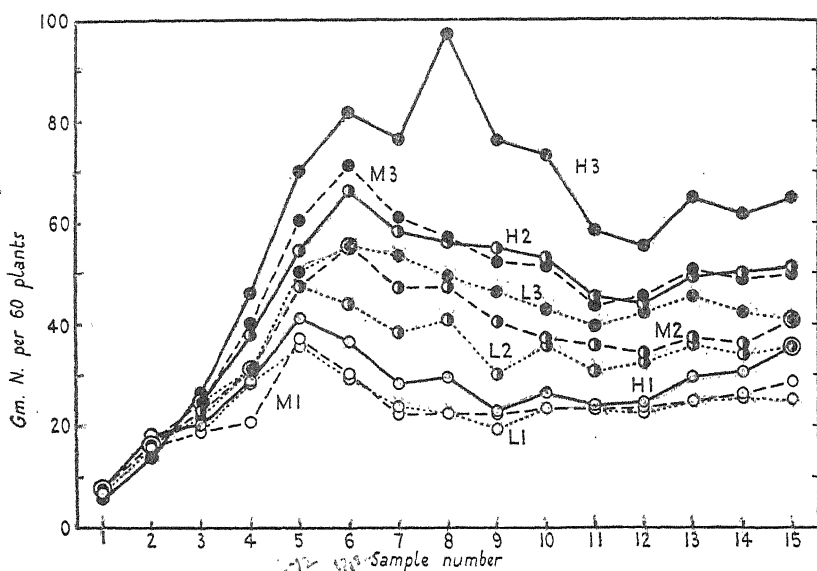


FIG. 4. Graph showing total nitrogen content of plants throughout season for all treatments.

water or nitrogen supply from the earliest samples up to the time of maximum leaf weight. In this respect these findings are in complete agreement with the results obtained by Gregory (6) on the effect of nitrogen on the growth and assimilation of barley.

### (c) *Nitrogen content of the plants.*

Nitrogen has been shown to play the predominant part in regulating the growth processes of plants. The results of systematic nitrogen determinations are shown graphically in Figs. 4 to 6. The total nitrogen content of the plant is presented for each treatment in Fig. 4 as grams of nitrogen per 60 plants. It is seen that at the first sample there are no differential effects of treatment, but with advancing age differences become apparent. All treatments show at first a rapid rise in nitrogen content, the three series with lowest nitrogen level (no added nitrogen) reaching a maximum simultaneously at sample 5 (December 3rd). The series at medium nitrogen level continue to rise to a maximum at sample 6 (December 17th), except for those receiving light watering, which reach their maximum at the fifth sample. The series at highest nitrogen level show differences with water supply; those with light and medium water reach their maxima at the sixth sample, but that with the highest level of both factors does not do so until sample 8 (December 31st). In general, the

group of curves resemble closely those in Fig. 2, for dry weight of the whole plant. As no independent replicates were available, no statistical

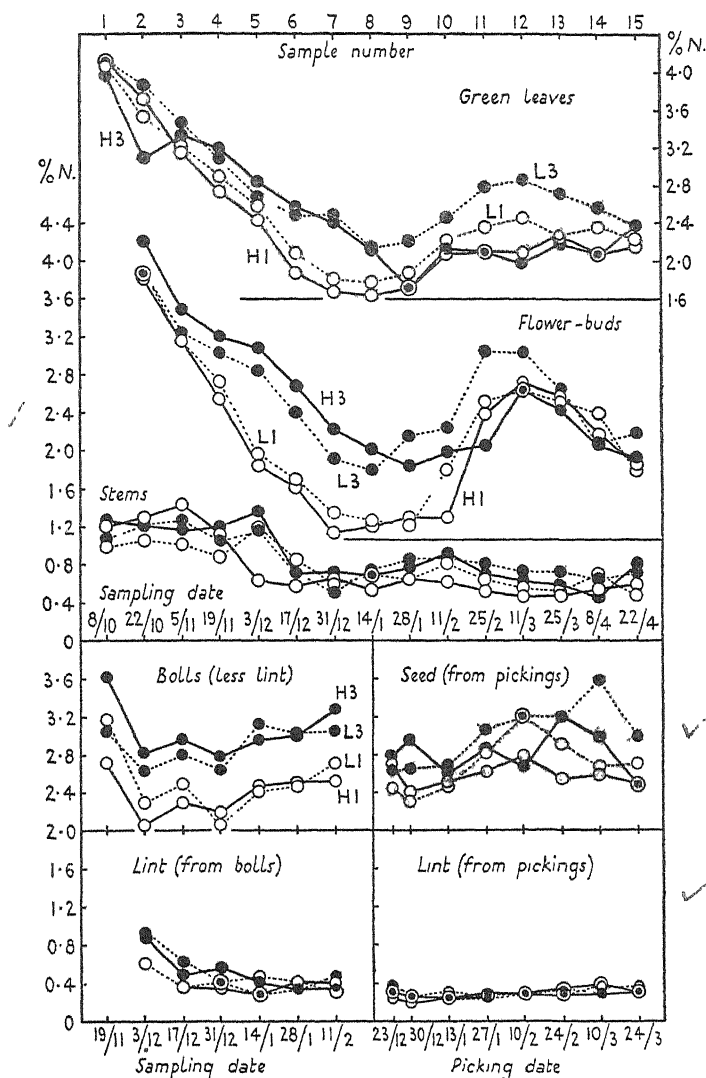


FIG. 5. Graph showing nitrogen percentage of dry weight for (a) green leaves, (b) flower-buds, (c) stems, (d) green bolls and lint, and (e) seeds and lint.

examination was possible. The effects of the single factors and their interactions resemble those already discussed.

The nitrogen contents are shown as percentages of the dry weights in Fig. 5 for the four extreme treatments, other treatments having intermediate values. In the green leaves there are only small treatment differences at

the time of the first sample. The nitrogen contents decline steadily with successive samples to minima at samples 6 and 7 in the series with low nitrogen, and at samples 8 and 9 in the series receiving high nitrogen. A subsequent rise occurs in all series. A delay in reaching the minimum value of nitrogen content of green leaves with increasing added nitrogen corresponds with the delay already seen in the time of minimum green leaf weight; a difference of three sampling periods is found in each case. Yet the actual time at which the minimum nitrogen content occurs is seen to antedate the time of the minimum leaf weight. Further, it is seen that the maximum point on the leaf weight curve in the series with low nitrogen level corresponds with a nitrogen content of approximately 2.5 per cent. The curves for the series with high nitrogen reach the same percentage content between samples 6 and 7, which again corresponds closely with the points of maximum leaf weight. It would appear, therefore, that a concentration of 2.5 per cent. of nitrogen represents a critical level at which dying off of old leaves overtakes production of new leaves.

The treatment effects on the percentage nitrogen contents of flower-buds are seen to be more marked than on the green leaves. At the first sample the nitrogen content is approximately the same as in the green leaves, and the treatment effects are not marked. The fall of nitrogen content is much more rapid in the low nitrogen series, and a minimum is reached at sample 7 (December 31st). The high nitrogen series show a delay of one period before the minimum occurs, and the level of nitrogen never falls so low in the flower-buds of these plants. Comparing these curves with those for flower-bud weights, it is again found that the maximum flower-bud production, which occurs at sample 4 in the low nitrogen series, corresponds with a nitrogen content of 2.5 per cent. The same nitrogen content in the high nitrogen series with light watering (L 3) occurs between samples 5 and 6, and in the high nitrogen series with heavy watering (H 3) between samples 6 and 7. These points are seen to coincide with the maxima on the flower-bud weight curves (Fig. 3). The apparent maximum flower-bud weight at sample 8 in the series receiving both maximal nitrogen and water supply (H 3) is obviously due to a sampling irregularity since high values occur in all parts of the plants for this sample (Figs. 2 and 3). The occurrence of maximal values of flower-bud weight at a nitrogen level of 2.5 per cent. would appear to be established with exactitude. The recovery in the leaf and flower-bud weights after the minimum has been reached is thus associated with a rise in percentage nitrogen content of the vegetative part of the plant, after removal of the mature bolls.

The nitrogen content of the stems shows little variation either with time or treatment. A minimum, not well marked, occurs at sample 7 at a time corresponding with the minima in the green leaves and flower-buds in

the series receiving no nitrogen. The accumulation of nitrogen, therefore, in the stems is not large. The absence of a differential effect due to treatments in this case indicates that the nitrogen in the stem is mainly in translocation, and that the fall in nitrogen content in the stem is due to the general fall in concentration in the leaves and flower-buds. The effects may be due in part to the increasing differentiation in the stem due to advancing age.

The effect of treatment on the nitrogen content of the bolls is very marked, the series at higher nitrogen level giving consistently higher values: the effect of water is small and inconsistent. The persistently high level of nitrogen in the bolls as compared with the rest of the plant is striking. The effect of treatment on the nitrogen content of the seed, though much less marked, is still evident. In the unripe lint from the developing bolls the nitrogen level slowly falls, and indications of treatment differences still persist, whereas the ripe lint is characterized by a uniformly low nitrogen content in all treatments.

The distribution of nitrogen within the plant at different stages of development is shown graphically in Fig. 6 for the maximal treatment (H 3); the other treatments all give similar results. The diagram shows that the stem at all stages contains relatively small proportions of the total nitrogen. At the time of the third sample the major portion of nitrogen is present as green leaves, but, after this, the total nitrogen content of the bolls rapidly increases, reaching by sample 6 one half of the nitrogen then present in the aerial parts of the plant. From this point onwards the nitrogen in the vegetative parts declines owing to death of leaves. After sample 8 the nitrogen is found to be increasing progressively in the seed cotton. During picking, therefore, the major portion of the nitrogen is removed from the plant. The beginning of secondary vegetative growth leading to nitrogen accumulation is clearly seen after sample 12.

The distribution of the total plant nitrogen between the vegetative parts (green leaves) and the reproductive parts (flower-buds, flowers, and bolls) is shown in Fig. 7 for the four extremes of treatment. The curves are necessarily mirror images, since the stems have a low and almost uniform nitrogen content. The rapid fall of nitrogen content in the leaves is thus due to the rapid increase in the nitrogen in the reproductive parts. The nitrogen supply from the bolls is evidently drawn mainly from the leaves. *The treatments produce only small differences, indicating that the distribution of nitrogen within the plant is independent of the absolute number of flowers and bolls produced.* The inference to be drawn from this is clearly that the progressive development is conditioned mainly by factors within the plant. The maximum lag between the extreme treatments is seen to be rather less than one sampling period, which reflects the delays in maturation due to nitrogen application. The plant thus behaves as if

the whole process were predetermined. It is in fact controlled by onset of internal nitrogen starvation through the drain on nitrogen supply within the developing bolls.

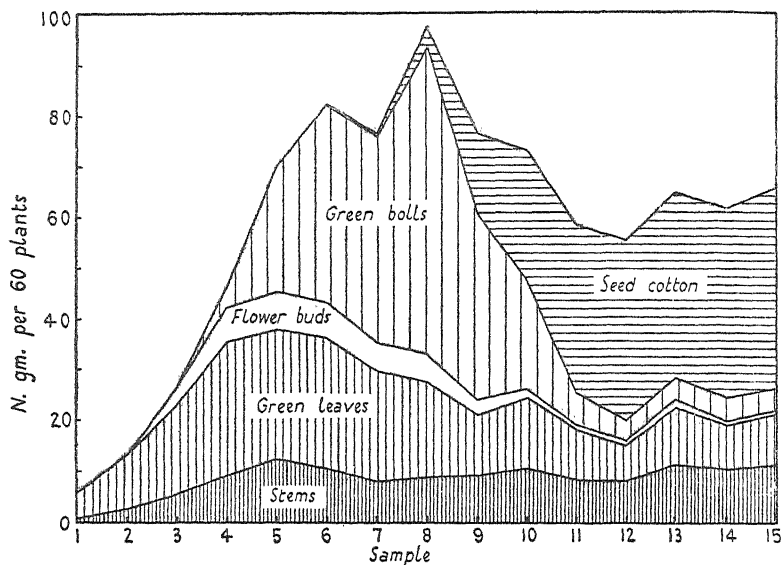


FIG. 6. Graph showing distribution of nitrogen within the plants of the treatment receiving maximal nitrogen and water supply ( $H_3$ ).

The effects of the treatments on the nitrogen economy of the whole plant are best seen from the diagrams in Fig. 8, in which the percentage nitrogen content of the whole plant is presented. Water supply has a negligible effect, but addition of nitrogen a marked effect on the rate of fall of the nitrogen concentration within the plant.

#### (d) *Uptake of nitrogen by the plants.*

From the dry weight data and the nitrogen content, the uptake of nitrogen has been calculated both as absolute and relative rates of uptake, and the effects of the treatments on these processes are shown in Fig. 9. The absolute rates of uptake (Figs. 9 *a* and 9 *b*) show a steady rise to a maximum between samples 4 and 5, and a rapid fall subsequently. The maximum occurs before the maximum leaf weight, and at the point of inflexion in the sigmoid curves for the whole plant weights. The transference of nitrogen to the developing bolls is most rapid at the time of maximum rate of nitrogen uptake (Fig. 7). *From this point of greatest demand for nitrogen by the plant, the rate of uptake of nitrogen from the soil rapidly declines.* The rate of nitrogen uptake is clearly influenced by nitrogen supply, but not markedly by the water supply. The relative rates of

nitrogen uptake (Fig. 9 *c* and 9 *d*) show a rapid decline from the first to the second period, after which they remain nearly constant, until the time

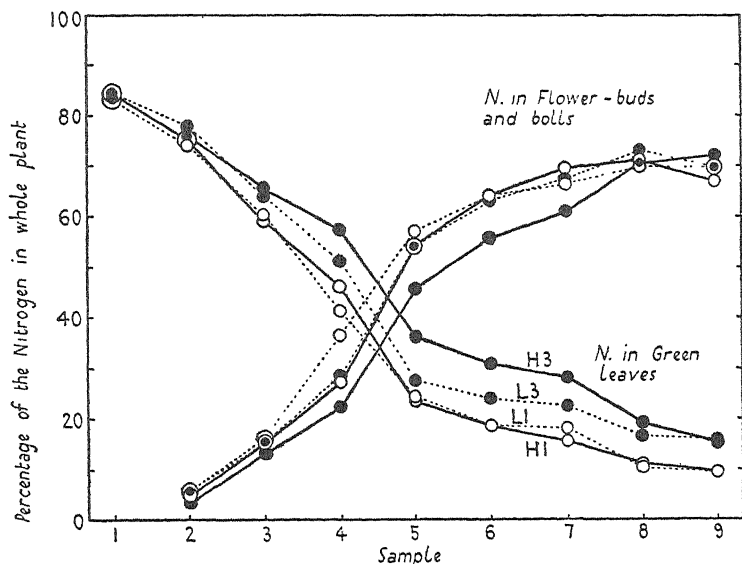


FIG. 7. Graph showing nitrogen in green leaves and in reproduction parts (flower buds and bolls) respectively as fractions of the total nitrogen in the plant.

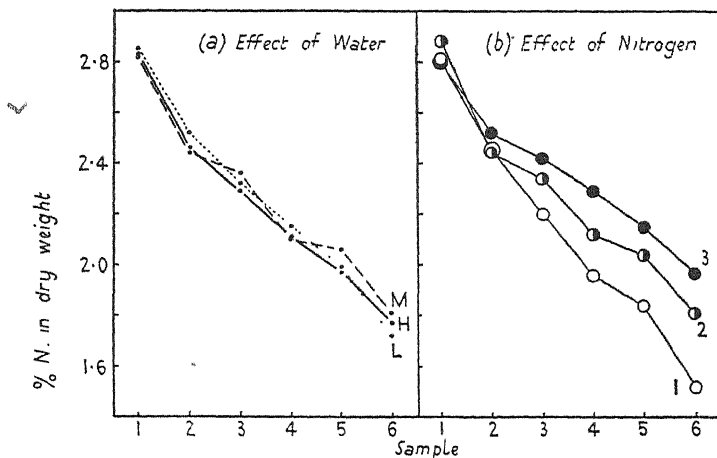


FIG. 8. Graph showing the percentage nitrogen in the whole plants for (a) effect of water supply and (b) effect of nitrogen supply.

corresponding with the maximum on the absolute uptake curves. After this, both absolute and relative rates fall rapidly to zero. The effect of treatment is not so marked on the relative basis, but nitrogen appears to

be more effective than water. These rate data are naturally subject to considerable errors of sampling.

Referring once more to the absolute rates of uptake in Figs. 9 *a* and

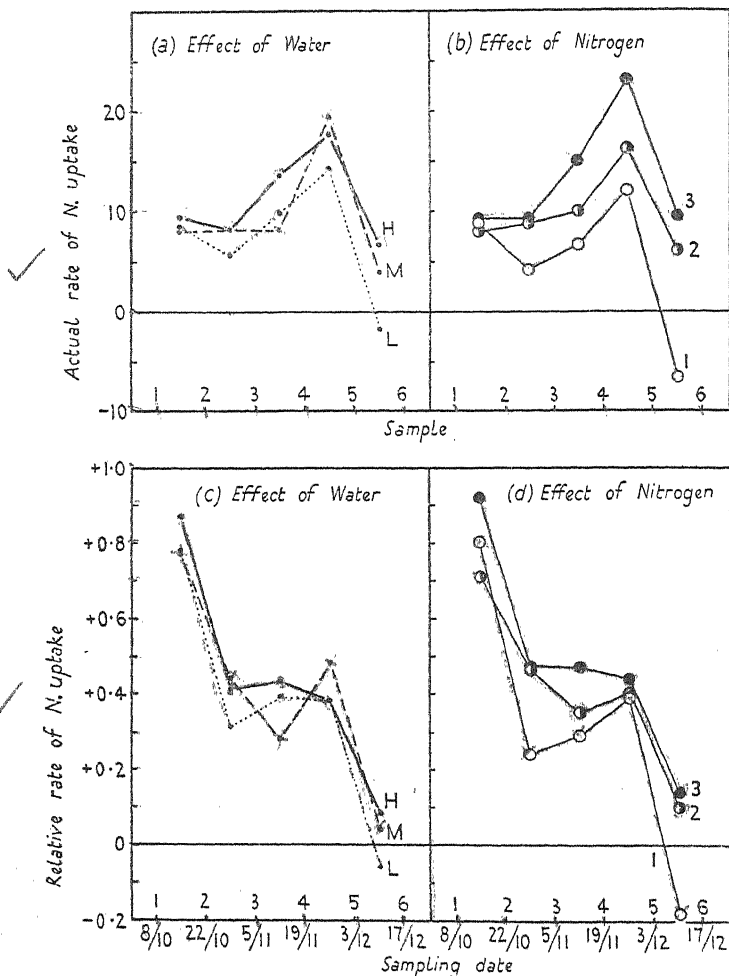


Fig. 9. Graphs showing effect of water supply and of nitrogen supply on the actual rate of uptake of nitrogen (*a*) and (*b*), and on the relative rate of uptake of nitrogen (*c*) and (*d*).

9 *b* it will be seen that from the time of the second sample the rate of uptake of nitrogen increases markedly with the soil nitrogen supply. Since the rate of uptake is the product of absorbing area and concentration, these differences may be due to differences in root size or in concentration or in both. The dry-weight data for the whole plant show no differences due to treatment at the time of the second sample (Fig. 2), and presumably, therefore, the root systems show no differences. Since high nitrogen level



is known to decrease root growth, it seems probable that the variations in the rate of uptake of nitrogen are due solely to variations in concentration of available nitrogen in the soil. The more rapid entry of nitrogen into the plants receiving high nitrogen is reflected in increased meristematic activity, leading later to increased morphological development of these plants.

# FINAL YIELD.

The final yields of seed-cotton of the separate experimental plots are given in Table V in a manner which shows the actual experimental layout.

TABLE V.

*Layout of Experiment and Yields (in Kantars per Feddan).*

(Nitrogen treatments are given in brackets before the yields.)

Water-duty.							
M	(2) 2.40	(1) 1.94	(3) 4.30	(2) 2.72	(1) 1.17	(3) 2.52	
H	(3) 3.58	(2) 3.25	(1) 1.35	(1) 1.53	(3) 3.62	(2) 3.12	
M	(3) 2.90	(1) 1.25	(1) 1.37	(2) 2.26	(2) 2.06	(3) 2.38	
L	(1) 1.53	(2) 1.90	(2) 1.97	(1) 1.41	(3) 2.17	(3) 2.16	
M	(3) 2.95	(2) 2.39	(1) 1.50	(3) 3.19	(1) 1.98	(2) 2.87	
H	(2) 2.68	(3) 4.27	(3) 4.16	(1) 1.78	(2) 2.37	(1) 1.41	
L	(1) 1.27	(1) 1.26	(2) 2.00	(3) 2.46	(3) 2.43	(2) 1.78	
L	(3) 2.20	(2) 2.12	(1) 1.36	(2) 2.12	(3) 2.28	(1) 1.44	
H	(3) 3.66	(3) 3.46	(2) 2.80	(1) 1.81	(1) 1.62	(2) 2.57	

The cumulative totals of the fortnightly pickings of each treatment are shown graphically in Fig. 10. The effects of the treatments and their interactions are very similar to those already studied in the flower data. The picking curves are all of the same type—sigmoid curves. The treatments with the lowest final yields have the highest early values, thus showing the delay in production of seed cotton at higher nitrogen levels. This delay has already been pointed out in connexion with the onset of the reproductive phase (Fig. 7). The points of inflexion of the curves occur between pickings 3 and 4 (January 13th to 27th). This delay of a fortnight between the times of maximum rates of picking corresponds to a similar difference in times of maximum weights of flower-buds, and, later, of green bolls, as is seen in Fig. 3.

The final weights of seed cotton for the various treatments are shown in Table VI.

The effects of both water and nitrogen are marked, nitrogen having the greater effect. The interaction also is very evident, and is of the same type as in the heights, flower-buds, bolls, and leaf weights. These effects are all highly significant statistically.

The results in Table VI were obtained from all the plants in the half-plots retained for yield measurements. All previous data on morphological

development were derived only from selected observation plants. Before definitive conclusions may legitimately be drawn from such data, their adequacy as representatives of the whole yield area must be considered. In order to do this the ratio of the yield of the observation plants to that obtained from the whole picking area has been calculated for each treatment (Table VII).

TABLE VI.

*Yields of Seed Cotton (Kantars per Feddan).*

		S. E. {			
		Water 0.22			
		Nitrogen 0.06			
		Interaction 0.18			
	Water	L.	M.	H.	Mean.
Nitrogen.	1	1.38	1.54	1.58	1.50
	2	1.98	2.45	2.80	2.41
	3	2.28	3.04	3.79	3.04
	Mean	1.88	2.34	2.72	2.32

TABLE VII.

*Ratio of Yields of Observation Plants to Whole Picking Area.*

(As percentage of general mean)					
	Water	L.	M.	H.	Mean.
Nitrogen	1	94	101	97	98
	2	107	98	106	104
	3	104	104	90	99
	Mean	101	101	98	100

It is evident that the variation in the individual treatment values is small, the maximum deviation being 10 per cent. Further, there is no evident relation between the discrepancies and the levels of the factors. The observation plants may therefore be regarded as a representative sample and conclusions based on measurements from these plants may legitimately be applied to the whole plots.

As the assimilation rate was found to be unaffected by nitrogen or water treatment, the successive leaf weights should give a measure of the amounts of carbohydrate material formed by the plants, and it should be possible to predict the final yield from the leaf weights throughout the season. Relative values of the mean leaf weight, expressed as a percentage of the basal treatment, agree closely with those for final yields, relative to the same basal treatment (Table VIII).

The correlation coefficient between mean leaf weight and final yield of seed cotton is +0.977, and therefore the final yield may be predicted with accuracy from the data of leaf weight obtained before picking commences. This method has been applied successfully to other Sudan experiments.

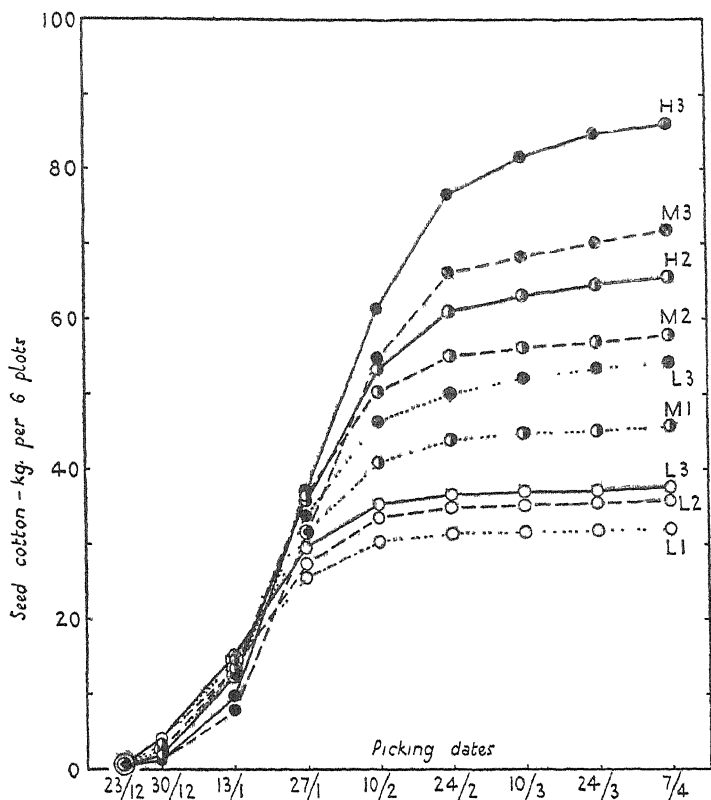


FIG. 10. Graph showing the total seed cotton picked up to each successive picking date for all treatments.

TABLE VIII.

Mean leaf weights, as percentage of basal treatment.					Final yields of seed cotton, as percentage of basal treatment.				
	Water.	L.	M.	H.		Water.	L.	M.	H.
Nitrogen	1	100	88	126	Nitrogen	1	100	112	115
	2	131	147	193		2	141	177	203
	3	157	198	265		3	165	220	281

An even simpler method of predicting yield depends on the correlation between height measurements and final yields. The correlation coefficients between height and final yield are:

for height on November 26th,  $r = +0.968$  (significance greater than  $P = 0.01$ ),

for heights on April 15th-16th,  $r = +0.979$  (significance greater than  $P = 0.01$ ).

Thus reliable estimates of yield under conditions such as existed in

this experiment can be obtained from height measurements a month before picking commences.

For completeness further relevant data on the effect of nitrogen and water supply on boll and lint characteristics are presented in diagrammatic form in Fig. 11. The time taken for bolls to mature after flowering (maturation period) was determined by labelling all the flowers on certain of the observation plants on each plot and recording the dates of opening of the bolls. Bolls subsequently found to be diseased were excluded from the averages. The healthy bolls were later used for lint-length measurements as described by Bailey (1). Samples were taken from all treatments at each picking for seed and lint weights, and for estimations of percentage weight of lint in seed cotton (ginning out-turn).

The effects of water and nitrogen on individual seed weight show that with increasing nitrogen the seed weight progressively increases, whereas a minimum weight is always obtained with medium water supply. The cause of this water effect is not clear. A similar effect is seen on the weight of lint per seed, and all these effects are statistically significant. The important practical quantity (ginning out-turn), percentage of lint by weight in seed cotton, is not significantly affected by water supply. It is reduced by increasing amounts of soil nitrogen (Fig. 11) doubtless because the extra nitrogen increases the weight of the seed without a corresponding increase in lint.

It would appear from the foregoing that the number of cotton hairs laid down remains constant in all treatments, and therefore the effect of treatment on lint weight must be largely due to variation in the length of lint. This is confirmed by the large effect of nitrogen on lint length, as shown in Fig. 11. The effect of nitrogen on the maturation period of the boll, as seen in Fig. 11, is such that higher nitrogen level leads to a slower maturation of the larger seeds produced.

The seed number per boll remains constant, and therefore in all treatments the larger seed weight obtained with higher nitrogen results in a greater weight of seed cotton per boll.

The effects obtained from gross yields of seed cotton apply equally well to the final yield of lint as is shown in Table IX.

TABLE IX.  
*Final Lint Weights (Rotts per Feddan).*

		S. E.		{		Water	23.7	
				{		Nitrogen	7.3	
				{		Interaction	12.7	
	Water.	L.	M.	H.		Mean.		
Nitrogen	1	133.6	156.5	163.2		151.1		
	2	195.0	243.6	266.3		235.0		
	3	224.4	300.2	359.3		294.6		
	Mean.	184.3	233.4	262.9		226.9		

The effects of the single treatments and their interactions are exactly similar to those seen in the weights of seed cotton, and are all found to be statistically significant.

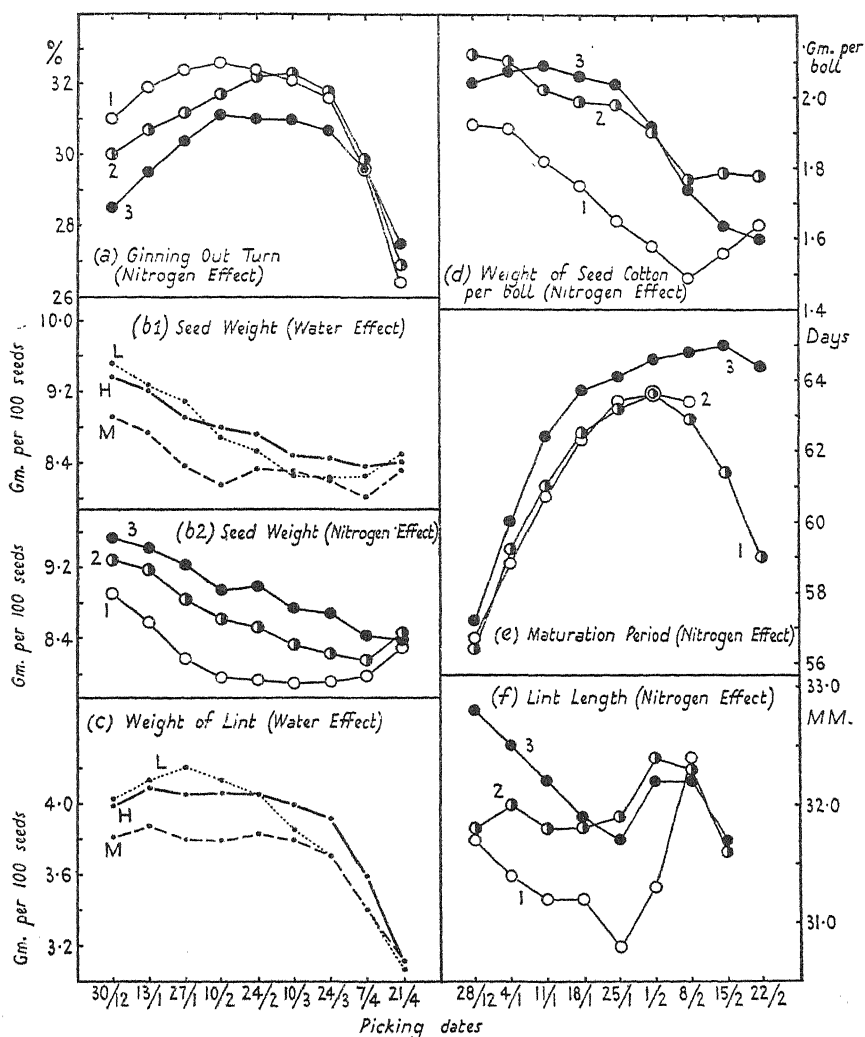


FIG. 11. Graphs showing the effects of (a) nitrogen supply on ginning out-turn (b1) water and (b2) nitrogen supply on weights of 100 seeds, (c) water supply on weight of lint per 100 seeds, (d) nitrogen supply on weight of seed cotton per boll, (e) nitrogen supply on maturation period of bolls and (f) nitrogen supply on lint length.

#### STATISTICAL ANALYSIS.

The tests of statistical significance quoted throughout this paper were made by an analysis of variance of the type illustrated in Table X for the final yield data.

TABLE X.

*Analysis Variance for Final Yield of Seed Cotton Weights.*

	Degree of freedom.	Mean squares.	Experimental 'Z' value.	Probability 5 % 'Z' value.
Water.				
Regression	1	6.4009	1.875	0.895
Deviation from regression	1	0.0181		
Error	6	0.1505		
Nitrogen.				
Regression	1	21.3290	2.912	0.719
Deviation from regression	1	0.2399	0.668	0.719
Error	27	0.0631		
Interaction.				
(a) Water regression v. nitrogen regression	1	2.5480	1.290	0.779
(b) Water regression v. nitrogen deviation	1	0.0032		
(c) Nitrogen regression v. water deviation	1	0.0053		
(d) Water deviation v. nitrogen deviation	1	0.0027		
Error	12	0.1929		

The limitations of the layout require separate estimates of error for the nitrogen treatments, the water treatments, and for their interactions. The nine water channels with three water treatments in random order give two degrees of freedom for treatment effects and six degrees of freedom for error, i.e. for the discrepancies between the similarly treated channels. Within each water channel there are three pairs of plots at random with similar nitrogen treatments. Each channel provides three degrees of freedom, giving twenty-seven for the error of the general nitrogen effects. The remaining sixteen degrees of freedom may be divided into four for the interaction of nitrogen and water, and twelve for the estimation of the standard error of this interaction.

The results for the three nitrogen or the three water treatments may conveniently be analysed further in view of the circumstance that the three treatments are equally spaced. One may express the average effect of a unit supply of water (or nitrogen) as a regression of yield on water (or nitrogen), and then ascertain how far the effects of the intermediate dressing deviates from the mean of the two extremes. If L, M, H represent yields from equally separated treatments, then the regression of yield on treatment is given by  $\frac{1}{2}(H - L)$  and the deviation from regression by  $M - \frac{1}{2}(H + L)$ . For strict proportionality between yield and level of treatment, the regression degree of freedom would absorb the whole of the variance. Any curvature of the line relating yield to the level of the factor concerned would show as a deviation from regression.

In the analysis of variance in Table X, and from the graphs in Fig. 12, it is clear that the mean yield curve in relation to water approaches a straight line, whereas the curve for nitrogen shows a considerable but

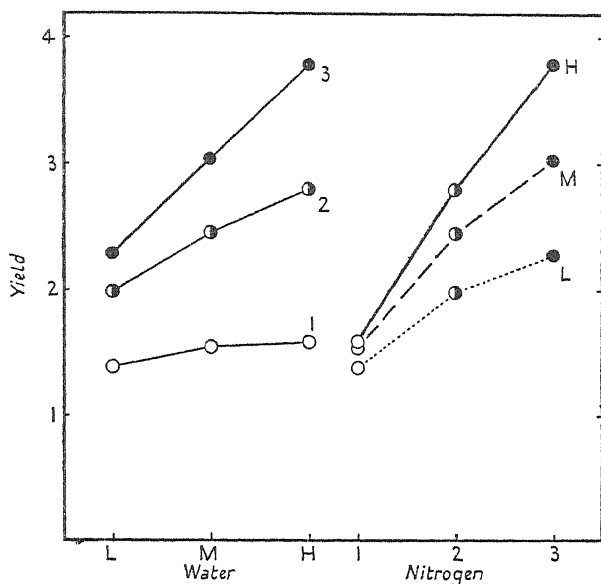


FIG. 12. Yield of seed cotton (kantars per feddan) as function of amount of water (L, M, H) and nitrogen (1, 2, 3).

statistically insignificant curvature. This close approach to proportionality justifies the consideration of the extreme treatments alone in many of the text-figures. It also shows that the experimental treatments were much below maximal, and that further increases in both water and nitrogen would have increased the yields considerably.

The changes of nitrogen effect with water supply and vice versa provide four degrees of freedom, which are set out fully in Table X. The average effect of water on yield (water regression) increases regularly with the amount of nitrogen, and the average effect of nitrogen on yield increases regularly with the amount of water. The effects of water or nitrogen on the shape as distinct from the slope of the curves for the other factor are quite trivial (Fig. 12).

The tabulation of 'Z' values provides a convenient means of summarizing the effects of water, nitrogen and their interaction on the various morphological characters studied. Table XI gives the 'Z' value for those measurements for which some treatment effect is definitely significant. The data are grouped according to the types of significant effects observed. They include both the regression and the deviation from regression for both water and nitrogen and the interaction of these regressions. The other

portions of the interaction were never significant. Table XII gives a similar summary for the dry weight of green leaves and of the whole plant at each of the fifteen sampling periods. Highly significant effects for water, nitrogen and their interaction were obtained consistently throughout the major portion of the growth period. The deviations from linear regressions which reached the significant level irregularly in one or two samplings out of fifteen are omitted.

The effects of treatment and of age on growth rates are shown as 'Z' values for the period October 8th to December 6th in Table XIII (a) and for the individual fortnightly periods in Table XIII (b).

TABLE XI.

*'Z' Values of Significances of Treatment Differences in Morphological Observations.*

Only 'Z' values above the 5 per cent. probability point are included. Those above the 1 per cent. probability point are given in heavy type.

Group (A)	Water supply.		Nitrogen supply.		Interaction of water regression and nitrogen regression.
	Regression.	Deviation from regression.	Regression.	Deviation from regression.	
Water duty, nitrogen application, and interaction all significant.					
Heights (Nov. 26th)	1.61	—	1.98	—	0.97
Final heights	1.57	—	2.00	—	1.09
Final flower number	0.95	—	2.02	—	0.92
Final healthy boll numbers	1.10	—	1.14	—	0.95
Final total boll numbers	1.25	—	2.29	—	1.30
Final yield, lint weights	1.74	—	2.60	—	1.42
Final yield, seed cotton weights	1.88	—	2.91	—	1.29
Group (B).					
Only water duty and nitrogen application significant.					
Node numbers (Nov. 26th)	0.98	—	3.44	—	—
Boll numbers (until Dec. 31st)	1.29	—	1.73	—	—
Seed weight	—	1.19	1.82	—	1
Group (C).					
Only water duty and interaction significant.					
Lengths of internodes	1.09	—	—	—	0.99

<sup>1</sup> No estimate of error available.



TABLE XI (*continued*).

Group (D).					
Only nitrogen and interaction significant.					
Flower numbers (to Dec. 31st)	—	—	2.15	—	1.15
Group (E).					
Only water duty significant.					
Percentage bolls shed	—	1.63	—	—	—
Weight lint per seed	—	1.23	—	—	1
Group (F).					
Only nitrogen application significant.					
Final node numbers	—	—	2.43	—	—
Weight seed cotton per boll	—	—	1.24	—	—
Maturation period of bolls	—	—	1.42	—	—
Lint length	—	—	1.74	—	—
Ginning out-turn	—	—	1.02	—	1
Group (G).					
No effects significant.					
Numbers seed per boll	—	—	—	—	—
'Z' values for 1% point	1.31	1.31	1.02	1.02	1.12

## DISCUSSION.

From the data presented it is possible to arrive at definite conclusions as to the interaction of water and nitrogen in controlling the growth of the cotton plant.

(a) *The Functions of Nitrogen and Water.*

The main function of nitrogen consists in initiating meristematic activity, whereas that of water is the expansion of the parts thus laid down. The total growth of the plant depends primarily on the rate of development of the leaf surface and the efficiency of the leaves produced. It has been seen that the variations in leaf growth rate are accounted for almost completely by variations in nitrogen supply, the effect of water being quite subsidiary. Furthermore, there is no evidence that variations in either water or nitrogen supply affect the net assimilation rates of the leaves. The physiological functions underlying growth are therefore seen to be largely independent of the primary processes of carbon assimilation.

The size of the plant is largely a measure of the rate of nitrogen metabolism and is independent of assimilation rate. The relative and absolute rates of uptake of nitrogen are therefore among the most important of the physiological reactions taking place in the plant. It has been seen that the relative rate of uptake follows the same course independently

<sup>1</sup> No estimate of error available.

TABLE XII.  
*Dry Weights of Green Leaves and Whole Plant: Significances of Treatment Differences.*

Sample.	Date.	Water regression.	Nitrogen regression.	Interaction: water regression and nitrogen regression.	Water regression.	Nitrogen regression.	Interaction: nitrogen regression and water regression.
			Green leaves.			Whole plant.	
1	Oct. 8th	—	0.45	—	—	0.55	—
2	Oct. 22nd	—	—	—	—	—	—
3	Nov. 5th	0.14	1.08	—	—	0.45	—
4	Nov. 19th	0.90	1.63	0.94	0.30	1.00	0.36
5	Dec. 3rd	1.51	2.10	0.87	0.52	1.02	—
6	Dec. 17th	2.56	2.36	1.35	1.24	1.70	0.56
7	Dec. 31st	1.56	2.66	1.11	2.40	2.21	0.35
8	Jan. 14th	1.02	2.42	1.22	1.79	2.20	1.41
9	Jan. 28th	1.10	2.45	0.93	1.84	2.08	1.44
10	Feb. 11th	1.52	2.15	0.91	2.30	2.29	1.08
11	Feb. 25th	2.06	1.85	0.96	1.85	2.41	1.66
12	March 11th	1.75	2.18	2.00	1.98	2.69	1.16
13	March 25th	1.35	1.89	—	2.19	2.61	1.01
14	April 8th	1.65	1.46	—	1.95	2.56	1.12
15	April 22nd	1.61	0.85	—	1.85	2.22	0.72
	5% 'Z' values	0.89	0.72	0.78	0.89	0.72	0.78
	1% 'Z' values	1.31	1.02	1.12	1.31	1.02	1.12

TABLE XIII (a).  
'Z' Values for Significance of Growth Rate Differences.

Water Nitrogen Time	Over whole period from October 8th to December 6th.		5% 'Z'.
	Relative leaf growth rate.	Relative rate total dry-weight increase.	
	0.71	0.13	0.89
	1.34	0.71	0.56
	2.41	1.98	0.49
	No interactions significant.		

TABLE XIII (b).

Over separate fortnightly periods from October 8th to December 6th.

Period.	Date.	Relative leaf growth rate.		Relative rate total dry-weight increase.		Net assimilation rate.	
		Nitrogen regression.	Deviation from nitrogen regression.	Nitrogen regression.	Deviation from nitrogen regression.	Nitrogen regression.	Deviation from nitrogen regression.
Sample 1-2	Oct. 8-21.	0.92	0.76	0.50	0.55	0.05	0.55
" 2-3	Oct. 22-Nov. 4	0.96	0.14	0.60	—	0.31	—
" 3-4	Nov. 5-18	1.03	—	0.30	—	—	—
" 4-5	Nov. 19-Dec. 2	1.05	—	—	—	1.17	1.05
" 5-6	Dec. 3-6	—	—	0.70	—	0.72	0.72
5% 'Z' value		0.72	0.72	0.72	0.72	0.72	0.72
		No interactions significant.					

of treatment, and that the absolute rate of uptake follows parallel courses for different levels of nitrogen. The distribution of nitrogen within the plant is substantially the same whatever the size of the plant. A self-regulating mechanism is clearly operating in some such manner as the following. The rate of uptake of nitrogen is determined primarily by the concentration of nitrogen in the soil solution, and the rates of development of the plant are proportional to the rates of inflow of nitrogen. In the early stages morphological development is not sufficiently rapid to reduce the internal concentration effectively, but, as growth is essentially exponential in nature, more and more regions are laid down in which the mobile nitrogen is synthesized. Thus, as has been seen, in all series with all treatments the internal concentration of nitrogen falls, the rate of decline being determined by (1) the concentration of nitrogen in the soil, and (2) the rate of development of the plant. The first of these factors is independent of the plant and can be controlled by manuring. So long as nitrogen supply is adequate, the second factor is determined solely by external factors. Eventually a point is reached in the life of the plant at which the rate of supply is inadequate to maintain the maximum rate of synthesis, and this point marks the beginning of internal nitrogen starvation. This condition is characterized by the appearance of symptoms of nitrogen starvation, and the time of onset is slightly delayed as nitrogen supply is increased. Evidence has been presented to show that this condition is associated with a definite level of nitrogen within the plant, viz.: 2.5 per cent. nitrogen in dry weight. The plant appears to be a victim of its own morphological processes. Once flower-bud production has begun, boll development inevitably follows, and the nitrogen and carbohydrate reserves of the plant are used up. It has been shown that rapid transference of nitrogen to the bolls occurs; Maskell and Mason (11) have demonstrated in addition a similar drain on carbohydrates. Mason (12) attributed the cessation of vegetative growth and augmented susceptibility to shedding to the drain on the carbohydrates, but the evidence indicates that nitrogen is also concerned. Cessation of vegetative growth is due to diversion of nitrogen from the growing points, and augmented bud shedding to competition among developing flower buds and bolls for available carbohydrate or nitrogen reserves.

Simultaneously with the rapid transference of nitrogen and carbohydrate to the bolls, the nitrogen uptake from the soil falls to zero. The sudden cessation of nitrogen uptake at this stage is certainly the most important fact established in this paper. Its cause cannot be precisely formulated from the experimental data since in this study roots have been omitted. On general grounds, however, it may be stated without much fear of error that this cessation of uptake is bound up with the drain of carbohydrate reserves by the developing bolls. As is well known carbo-

The bolls are found to exert dominating effect on the whole plant when their development begins. This effect results in cessation of apical growth of the main stem and in the stoppage of nitrogen uptake from the soil, presumably through checking of root growth. The nitrogen supply to the plant as a whole is interrupted just at the time of the most serious drain on the nitrogen reserves of the plant by the developing bolls. It is suggested that the cessation of root growth operates through interference with the carbohydrate supply to the roots.

The type of interaction between the factors was such that the increase in response to either factor increased with a higher level of the other.

The practical importance of the results, particularly in connexion with the necessity for obtaining favourable early growth of the crop, is stressed.

The author wishes to record his indebtedness to Dr. F. G. Gregory of the Imperial College of Science and Technology who proposed the original experiment, and to whom the interpretation of the results obtained is in large measure due, and to Dr. E. M. Crowther of Rothamsted Experimental Station for help in the statistical work.

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# The Effect of Intercellular Pressure on the Suction Pressure of Cells.

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THE modified method of measuring suction pressure of the cell is to strip off the epidermis with one row of mesophyll cells adhering to it, vide (1). Using such mesophyll cells, which show no signs of mechanical injury, the concentration of cane sugar is then determined in which they show neither an increase nor a decrease in surface area as observed under a high-power lens.

This measures the water-absorbing capacity of the cell at the time of observation. When the water deficit in a leaf is reduced to zero, i.e. the leaf can absorb no more water, the suction pressure of the cell-system must be zero. Mesophyll cells of fully turgid leaves have, however, a considerable suction pressure when observed in strip preparations, as will be shown below. It is therefore suggested here that the positive suction pressure value which is exhibited by cells stripped from a leaf whose water content has reached a maximum is an indication that the tissues of a leaf offer mechanical resistance to the distension of the cell by internal hydrostatic pressure. In other words, the suction pressure equation requires to be amended by the introduction of an additional value which, with the wall pressure, must be subtracted from the osmotic pressure in any indirect or theoretical estimation of suction pressure.

In one paper Ursprung and Blum (2, p. 53) suggested that cells might be prevented from fully expanding by intercellular pressures. The sum of these pressures they represented by  $A$  and equated

$$S_z = S_i - W - A$$

where  $S_z$  equals suction pressure of the cell,  $S_i$  the osmotic pressure or suction pressure of the cell contents (Saugkraft des Zellinhaltes)<sup>1</sup> and  $W$  the wall pressure. This factor has been omitted from the basic equation

<sup>1</sup> This expression is confusing and objectionable.

in all of their subsequent papers, and no reference to it has found a place in the literature of the 'water-relations' of cells of higher plants. It appears to have been generally assumed that this factor is of negligible importance, and such an assumption is to some extent justified by the extensible nature of the walls of cells of many plant tissues, those normally growing immersed in water or in conditions of water scarcity being, apparently, exceptions. The presence of intercellular spaces has also undoubtedly caused many to assume that the intercellular pressures are of small importance. That the cell-wall is not capable of deformation to the extent of entirely obliterating these spaces is shown by their maintenance in leaves saturated with water.

Water is drawn into the cell by the dissolved substances in the sap exerting osmotic pressure, the action being diminished by the resistance the cell-wall offers to distension. This resistance is partly that of the wall itself and partly that of the pressure upon it of neighbouring cells and of the vascular tissue. If a cell is removed from the plant the mutual pressure of neighbouring cells is reduced to zero; the wall pressure to be subtracted from the osmotic pressure is then solely that due to its stretched wall. It is possible at present to determine only the *maximum* value of the intercellular pressure; that it has a positive value between the extremes of full turgidity of the tissue and the stage at which the tissue wilts is a natural deduction.

#### METHOD.

Leaves have been chosen which allow the epidermis to be stripped in such a manner that intact mesophyll cells remain attached to it. As has already been demonstrated (1) the use of such tissues provides preparations thin enough for convenient microscopic study of intact cells which have not been exposed to the osmotic and chemical action of sap exuded from neighbouring wounded cells. When the cells of sections are employed exposure to such sap vitiates the results obtained.

An Iris leaf is cut into 2-inch lengths and the pieces floated on distilled water in Petri dishes kept at constant temperature in the dark. The suction pressure of the subepidermal mesophyll cells from one of these lengths of leaf is measured at convenient intervals of time. It might be expected that the cells would become saturated fairly quickly under these conditions where one surface and the cut ends of each leaf are in contact with water, and the other surface is exposed to an atmosphere which must have a high water content since the water surface is large compared with the volume of air within the Petri dish, that volume being reduced to a minimum by filling the dish almost to the top. The suction pressure decreases with time under these conditions, but the rate of decrease soon falls off and the suction pressure in no case falls to zero, even after three days (as is shown in Table I).



TABLE I.

*Suction Pressure of Mesophyll Cells Torn at Intervals from Portions of a Leaf Floating in Water.*

*Iris asiatica.*

Time.	Cane sugar (M).
0 min.	0.38-0.39
45 "	0.36-0.37
2 hours	0.34
3 "	0.33
5 "	0.32-0.33
22 "	0.31
3 days	0.29-0.30

*Iris florentina.*

Time.	Cane sugar (M).	
	1st leaf.	2nd leaf.
0 min.	0.384-5	0.385-6
15 "	0.380	—
30 "	0.375	0.370
60 "	0.375	0.355
2 hours	0.360	—
3 "	—	0.330
4 "	0.340	—
6 "	—	0.330
8 "	0.330	—

The fall in suction pressure was followed with more finely-graded sugar solutions for various species of *Iris* as is shown in Table II.

TABLE II.

*Original Suction Pressure (A) and After Absorption of Water During 5 hrs. (B).*

	Cane sugar (M).	
	A.	B.
<i>I. pallida</i> . . . . .	0.38	0.30-1
<i>I. jacquesiana</i> . . . . .	0.36-7	0.28
<i>I. germanica</i> . . . . .	0.42	0.30-1
<i>I. florentina</i> . . . . .	0.38	0.30
<i>I. variegata</i> . . . . .	0.38-9	0.30
<i>I. neglecta</i> . . . . .	0.40-1	0.30-1
<i>I. olbiensis</i> . . . . .	0.41	0.30
<i>I. asiatica</i> . . . . .	0.38-9	0.32-3

Similar tests were carried out on leaves from certain dicotyledonous plants, the leaves being allowed to float on water for five hours to attain an approximate equilibrium. The results are given in Table III.

The suction pressure of mesophyll cells taken from highly turgid leaves which have ceased, or almost ceased, to absorb water is remarkably high, and would appear to be a measure of the pressure exerted on a cell

by the surrounding cells within the turgid leaf. This pressure conceivably decreases as turgor decreases, but for want of a suitable method its value at other degrees of turgor of the leaf has not yet been measured. It seems

TABLE III.

*Suction Pressure of Mesophyll Cells, (A) Before, and (B) After Absorbing Water for 5 Hours.*

	Cane sugar (M).	
	A.	B.
<i>Isatis tinctoria</i> . . . . .	0.30-0.31	0.24
„ <i>Boissertianum</i> . . . . .	0.31-0.32	0.24-0.25
<i>Valeriana Phu</i> . . . . .	0.34-0.36	0.22-0.24
<i>Sidalcea malvaeflora</i> . . . . .	0.34-0.36	0.22-0.24

probable, however, that until this intercellular pressure falls to zero it considerably reduces the suction pressure. The suction pressure equation should therefore be written, as was first adopted and then given up by Ursprung and Blum, in the form

$$Sp = Op - (Wp + A)$$

where  $Sp$  equals the effective suction pressure of the cell within the plant,  $Op$  the osmotic pressure of the cell sap,  $Wp$  the pressure of the extended cell-wall, and  $A$  represents the intercellular pressure. It now appears that this factor,  $A$ , represents a pressure of considerable size.

## SUMMARY.

Mesophyll cells *removed* from a portion of a leaf which has been floating for some hours in water, and which therefore would be expected to be fully turgid, show a considerable suction pressure which may be as high as eight atmospheres. This suction pressure is due to the fact that the expansion of individual cells is resisted by an intercellular pressure, i.e. the mutual pressure of surrounding cells.

The suction pressure equation should therefore take the form of  $S = Op - (Wp + A)$ , where  $S$  is the suction pressure,  $Op$  the osmotic pressure,  $Wp$  the wall pressure of the cell, and  $A$  the sum of the intercellular pressures.

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# An Analysis of the Influence of Temperature during Germination on the subsequent Development of certain Winter Cereals and its Relation to the Effect of Length of Day.

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With Plate XX and twelve Figures in the Text.

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## I. INTRODUCTION.

WINTER varieties of cereals may be regarded as annuals, since, sown at the normal time, they complete their life-history within twelve months. When sown in spring, however, they behave as biennials, making great vegetative growth in the first season, but flowering only after the winter. This suggests that, in these varieties, something in the winter conditions is necessary for the inception of reproduction, whereas closely related 'spring' forms differ from them in the ability to ear within a few weeks of spring sowing, and in their lack of hardiness to winter conditions.

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These differences seem to be inherent, and Linnaeus classified summer and winter wheats as distinct species, but Darwin (5) considered that the experiments of Monnier showed that they should not be so distinguished. Gassner (8) has stated that summer and winter rye were bred originally from the same population.

Whatever may be the relationship between the two classes of cereals, it is certain that winter forms are dependent for the completion of their life cycle on the influence of some of the factors associated with autumn sowing, whereas corresponding summer forms are independent of these. Such factors would appear to be as follows—(a) a prolonged rest period, (b) a long period of vegetative growth, (c) low temperature during the early stages, and (d) short days during earlier stages followed by increasing day-length.

Maximov (15) has pointed out that neither (a) nor (b) can be the determining factor. He quotes the work of Grebennichev, who sowed a winter variety so late in the autumn that actual germination did not occur until the spring—in this case the time taken to flower was short, and there was no long vegetative period. There is no record of the actual date of germination, but presumably it would occur with the first warming of the soil, while days were still short and temperature liable to fall again to freezing-point. Maximov also considers that his own work in conjunction with Pojarkova (17) shows that a long rest period is not the essential requirement for flowering. They grew winter varieties in a greenhouse in the winter, when vegetative growth continued throughout, until returning long days brought flowering. The initiation of the reproductive phase of winter cereals is presumably the result of the plant's response to either low temperatures during early stages or to photoperiodic variations.

It is an established fact that a low temperature, especially when applied during early stages does promote flowering in many plants. Maximov states that this effect was first used by a Petersburg gardener named Gratshev, who, finding climatic conditions unsuitable to the flowering of artichokes, was able to induce flower formation by cooling the plants. Miller (18) was able to keep cabbages for two and a half years in a continuous vegetative condition by growing them in a warm greenhouse, whereas transfer to a cool house immediately induced shooting. Gutzeit (9) and Thompson (28) found that germination at a temperature of about 4° C. induced shooting in beet and celery respectively. Thompson records that actual freezing prevents ultimate flowering; Maximov and Pojarkova have also noted its harmful effect. Appel and Gassner (1) had observed that in the case of certain cereals a high germination temperature gave unhealthy plants with excessive leaf production which were unable to produce seed. To this they attribute the failure of cereals from temperate regions to grow in the tropics. Following this work, Gassner in 1918 investigated

thoroughly the effect of germination temperature on both summer and winter varieties of cereals (8). He used temperatures from 1°C. to 26°C. for germination and sowed in the spring. With his winter varieties he found (1) The lower the germination temperature, the shorter the time required to flower; (2) The earlier in development the exposure to low temperature the greater the effect.

With closely related summer varieties he obtained no effect, except in a few cases where a slight acceleration of flowering followed germination in the cold. Kidd and West (11) list this effect with other cases of what they term 'physiological pre-determination' in which the treatment or condition of the seed influences indirectly the future development of the plant.

Gassner (8) argued from his results that winter varieties differ from their related summer varieties in having a definite 'cold requirement' (Kältebedürfniss) which must be satisfied before flowering can take place. He considered that this was correlated with the cold-resistant properties of such varieties.

Maximov and Pojarkova (17) found that the date of sowing profoundly affected the potency of this 'temperature after-effect'. Later Pojarkova (19) working alone, found (1) that with early sowing, December to March, ear formation eventuates in the greenhouse without being in any way influenced by the germination temperature, (2) that cold germination begins to be effective only when sowing is so late that fruiting begins to be suppressed, (3) that with very late sowing (after June) cold germination loses its effectiveness.

The phenomenon to which Garner and Allard (7) have given the name photoperiodism, the response to the relative length of day and night is considered by Maximov to be of equal or greater importance in determining the behaviour of winter cereals. Garner and Allard (6) have found that plants may be divided into two categories (with certain exceptions) according to their reaction to the length of day.

The winter cereals under consideration are all 'long-day' plants—flowering in a day-length which obtains in the temperate summer, and growing vegetatively when the day is less than twelve hours' long. Maximov holds that the most important point is to sow at such a date that a long day prevails at the time of 'shooting', but cold germination also speeds up flowering provided that the days are long at this time. He disagrees with Gassner's concept of a 'cold requirement' in the plant, and postulates an antagonism between tendencies to vegetative and reproductive growth. For winter varieties he suggests that the tendency to vegetative growth so far predominates that an outer stimulus is required to initiate reproduction, and this stimulus is in some way provided by cold germination. The cold after-effect also includes a diminution in the production of vegetative organs, including leaves and roots. These differences are held

to be indirect rather than direct consequences of germination temperature. The direct effect is a more rapid onset of the reproductive phase, resulting in a different distribution of reserve food materials.

Rasumov (21) has revealed a photoperiodic after-effect similar to the temperature after-effect, when a particular day-length is applied only at the beginning of growth.

It is obvious from the existence of these 'after-effects' both of temperature and day-length that we are dealing with an agency whose effect remains for some time after the application has ceased. Maximov points out that at the time of action of the stimulus no flower initials are present and therefore no direct effect is possible: a self-propagating chemical agency must therefore be postulated. At the same time it must not be forgotten that there is a direct lineal connexion between the flower initials and the cells subjected to the stimulus. And again, if flower initials were present at the moment of stimulation, the stimulus could not be held responsible for their initiation. It seems that here lies the weakness of much of the very informative work that has already been carried out—workers have regarded 'flowering' as connoting an *external* manifestation of the existence of flowers, such as 'shooting' and ear emergence, and insufficient attention has been paid to the earlier stage of differentiation of flower primordia.

Klebs (12) working on *Sempervivum* notes three stages in flower production. (1) The production of a condition of ripeness to flower, (2) the formation of primordia, and (3) the development of floral structures and their expansion. This concept of flower production is obviously applicable to all plants. Most workers on problems relating to flowering have confined their observations to the third phase, but it is clear that the two first are of paramount importance in elucidating the relationship between external conditions and flowering.

The questions involved in the problem of flowering in winter cereals may be posited in the following terms: (1) What is the morphological condition of plants which fail to flower in consequence of spring planting? (2) Does failure to flower connote a failure of flower differentiation or are flower initials formed which do not continue their growth? (3) Do all factors which tend to inhibit flowering operate in the same way? The present investigation has been carried out with the object of elucidating these questions, and to find how far failure to flower may be correlated with the vegetative condition of the plant, including under this term its chemical composition. In view of these considerations efforts have been made to modify as much as possible the vegetative conditions of the plant, thereby among other effects altering the ratio of carbohydrate to nitrogen which has been regarded as a determining factor in flower production. This has been achieved in the following ways: (1) by nitrogen starvation

(known to increase carbohydrate content and reduce vegetative growth),  
(2) by potash starvation (known to increase nitrogen content, in some cases to reduce carbohydrate, and to leave meristematic activity unaffected,  
(3) by artificially shortening the daily exposure to light.

Plants have been grown under the conditions stated after germination at a high (18°C.) and at a low (1°C.) temperature, and kept under close observation during the whole of their growing period.

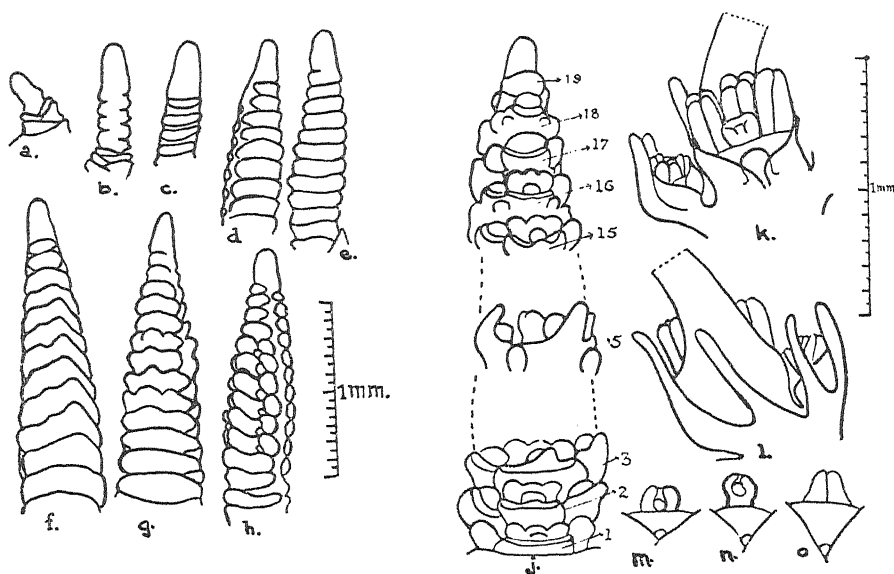
## II. MORPHOLOGY OF THE CEREAL PLANT.

Before setting out the results obtained, a brief reference will be made to the morphology of the plants under consideration. The mature, normally-grown, cereal plant consists of a main axis bearing alternate leaves on an elongated stem which is terminated by an inflorescence, and a number of basal branches, or tillers of similar structure. These tillers arise in the axils of the lowest leaves of the main axis or as branches of other tillers. The elongated stem carrying the ear consists of the youngest five or six internodes—below this the nodes are crowded together, and numerous adventitious roots arise. At an earlier stage before the younger internodes were produced, the plant has passed through a 'rosette' stage, which is purely vegetative, and it is not until conditions necessary to flowering obtain that extension growth begins. When these conditions fail, as in the case of spring-sown winter cereals this rosette stage is maintained indefinitely and more and more tillers are produced, until the plant resembles a tussock.

Young plants of barley and rye (from four weeks old) were carefully examined before they reached the stage of stem elongation, the main axis being dissected to expose the apical meristem. Text-fig. 1 *a* shows the apex of a barley plant (four weeks old) from which all expanded leaves have been removed. At the base are five lateral growing points which appear to be those of leaves, since a slight degree of foliar expansion is evident, and beyond these a spike-like growing point. Later this bears two rows of alternating ridges, each ridge embracing rather more than half the circumference. These appear to be different structures from the leaf initials below them, and when seen in profile, as in Text-fig. 1 *b*, each was divided into two parts, suggesting a leaf and its axillary bud. In Text-fig. 1 *c* the same spike is seen, with the surfaces of the ridges exposed. Later (Text-fig. 1 *d-h*) the ridges increase in size and in number, and the double appearance is lost—apparently the axillary structure has grown at the expense of the subtending bract. The undifferentiated portion at the tip remains about the same length, i.e. 0.25 mm.—in other words, it extends at the tip as rapidly as it produces new ridges.

The stages by which the lateral ridges become differentiated as groups of flower-bearing spikelets has been described by Schuster (22). Observations on later stages are also shown in the diagrams (Text-fig. 1).

The early stages of ear development in winter rye are similar to those described for barley; dissection of plants between four and six weeks old



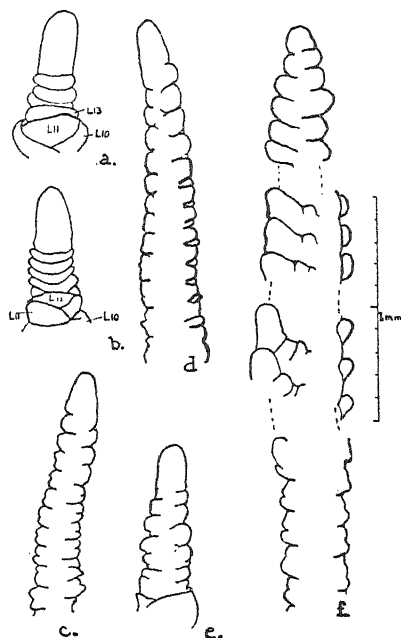
TEXT-FIG. 1 *a-o*. Plumage Archer barley. *a-j*. Early stages in differentiation of the spike. *k* and *l* adaxial and abaxial views of a spikelet group, showing stamens and gynoecium of a central flower, and one lateral sterile flower. *m*, *n*, and *o* stages in growth of gynoecium.

reveals a long thin meristem with two ranks of lateral ridges, which are more numerous than in barley, but show the same double structure (Text-fig. 2).

On the axis shown in Text-fig. 2 *a* there are eleven definitely leaf-like structures, and the nature of the ridges that follow cannot be determined by observation only. It is known, however, that plants similarly treated had an average of thirteen green leaves on the main axis below the ear, and if this plant had continued to grow, and had had thirteen leaves, then two of the indefinite ridges would have been leaves, and four, in addition to any subsequently formed, would have produced spikelets. On the other hand, Text-Fig. 2 *b* shows a growing point, at about the same apparent stage, from a plant treated in such a way (warm germination) that about twenty-two leaves may be expected to occur on the axis before earing. As ten leaves have been removed, initials of twelve more are required, and for these ten initials have been laid down at the time of examination, (remembering the row of alternating ridges on the hidden side of the spike). Thus of two apices of similar appearance, one has a number of ridges



destined to produce flowers, while the other has not yet laid down enough to provide for the necessary number of leaves. In this investigation the length of this long apex and the number of ridges it bears have been used to characterize the different stages of development. Measurement has been made from the last removable leaf to the tip of the apex, and is termed 'spike-length' throughout this paper. As in barley, the first visible sign of flower development is the swelling of certain ridges. This occurs as a rule very near the tip, and frequently there is a brief stage in which new leaves are being expanded at the base while upper ridges are beginning to produce floral structures.



### III. EXPERIMENT OF 1931.

A preliminary experiment was carried out in 1931 with the object of determining the best method of experimentation for 1932. The scheme, therefore, was made comprehensive in scope at the expense of reducing the number of replicates in each series.

Winter and summer varieties of barley, oats, and rye<sup>1</sup> were grown in water-cultures in an unheated greenhouse, and also in soil contained in 8-inch earthenware pots which were kept out of doors standing on gravel. The plants had been germinated at different temperatures (Table I) and planted out when the coleoptiles were about 1 cm. long. The date of planting was regarded as the initial date of each experiment, and sowing was timed so that all plants reached approximately the same size on the day of planting. A series of experiments, with successive planting dates throughout the summer, varied the day-length obtaining during the period of growth, but no variation in manuring was employed.

In this preliminary work a small number of replicates was used so that the scope should not be limited. Observations of tillering and spike-development were made on samples taken at intervals through the summer. The results obtained were confirmed by experiments in two

<sup>1</sup> Winter Barley, *Hordeum hexastichum* F. 112. Summer Barley, *Hordeum vulgare* 'July'. Given by Dr. E. S. Beaven, Warminster. Winter Oats, *Avena sativa*, 'Grey Winter', Summer Oats 'Abundance' supplied by N.I.A.B. Cambridge. Winter Rye, *Secale cereale*, 'Petkus Winter'. Summer Rye, 'Petkus Summer', supplied by Messrs. Haage and Schmidt, Erfurt.

TEXT-FIG. 2 a-f. Petkus winter rye; early stages in differentiation of the spike.

subsequent years, and will therefore be presented in a summarized form only.<sup>1</sup>

(1) In no summer variety was any temperature after-effect manifested, but short days had a marked effect in preventing flowering.

TABLE I.  
*Scheme for 1931 Experiment.*

Planting date.	Average day-length during first 8 weeks in hours.	Range of temperatures used for germination.		
		Oats.	Barley.	Rye.
May 11th	16.15	1°, 18°	1°, 5°, 12°, 18°, 25°	—
May 18th	16.27	—	—	1°, 5°, 12°, 18°, 25°
June 22nd	15.85	—	1°, 18°	1°, 18°
July 27th	14.03	1°, 18°	1°, 12°, 18°, 25°	1°, 12°, 18°, 25°
Nov. 4th	8.40	1°, 5°, 12°, 18°, 25°	1°, 18°	1°, 5°, 12°, 18°, 25°

(2) The variety of 'winter' barley used was capable of early flowering after spring planting, and in this respect should be denominated a 'spring' variety. For this reason no temperature after-effect was manifested.

(3) Grey 'winter' oats was also capable of flowering after spring planting, but germination at 1° C. lead to ear exsertion two weeks earlier than did germination at 18° C.

(4) Spring-planted Petkus winter rye, which failed to flower after germination at 12°, 18°, and 24°, flowered in thirteen weeks after germination at 1°, and in about fifteen weeks after germination at 5° C.

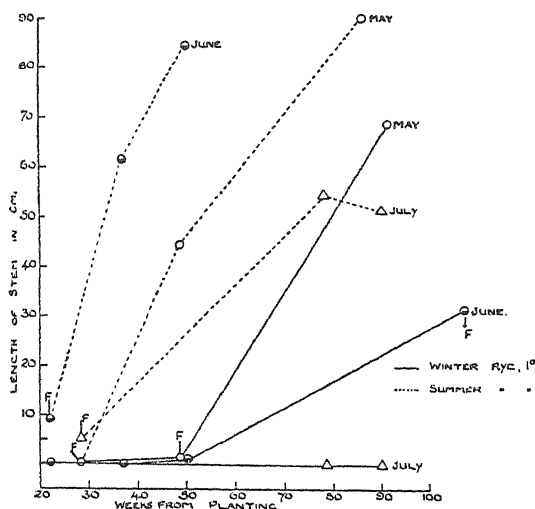
(5) This effect was not obtained in the short days of August and September; flowering now failed after germination at any temperature.

(6) Sowing in early winter gave rise to plants which through the period of very short days grew slowly without tillering and with no differentiation of flower initials. In February (ten-hour day) tillering began, and flower differentiation appeared late in March, giving rise to ears which on May 16 were on the point of exsertion. Summer varieties so planted developed in the same way. Thus the minimum day-length allowing differentiation is about ten hours.

Observations were also made on the rate of stem growth, which were not repeated in subsequent years, and are therefore given in full.

<sup>1</sup> Full details of these experiments, and other points which are excluded from this paper on account of limited space may be seen in the Library of London University (Thesis for Ph.D. degree, 'An Analysis of the Influence of Temperature during Germination on the Subsequent Development of Certain Winter Cereals, and its Relation to the Effect of Length of Day', by O. N. Purvis. April, 1934).

*Growth of stems.* Evidently stem growth is in some way correlated with flowering. In cases in which flowering failed, no elongation of internodes took place at all, and in the plants grown throughout the winter,



TEXT-FIG. 3. 1931 experiment. Winter and summer rye. Effect of planting date on progress of stem length.

elongation did not begin until after the inception of flowering in March, and the internodes involved were those between leaves which were then green.

In summer rye germination temperature was without effect on either flowering or stem elongation, and Text-fig. 3 shows the progress of stem length after different sowing dates: in each case the figure given is the mean for all germination temperatures. Sowing date is seen to have a considerable effect on stem length, indicating its dependence on seasonal climatic variations.

It can be seen that stem elongation is most rapid in June-sown plants, and that in the July sowing it is considerably reduced. Clearly this is not entirely a photoperiodic phenomenon, since day-length is slightly shortened for the whole period of the June sowing, and more so for the period during which stem elongation is proceeding. On the other hand, during the first few days of growth, the June 22nd planting was subjected to the maximum day-length ( $16\frac{1}{2}$  hours).

The increased stem growth rate of the June planting may then be attributed to (a) the after-effect of very long days during the first week, or (b) the higher temperature during the whole period of growth. It is quite obvious that as stem elongation is a growth phenomenon its rate must

increase with rising temperature. On the other hand, there is evidence that it is also controlled by length of day, since (a) the rate of elongation is depressed by planting in July, and (b) in plants grown during the two succeeding years in artificially shortened days the stems failed to elongate. Probably both long days and high temperatures are requisite for elongation. Stem elongation cannot take place under conditions unfavourable to flowering, e.g. in the winter variety it only occurs in plants which have been germinated at low temperatures, and grown in long days.

Table II gives stem lengths of winter rye after 1° germination.

TABLE II.

*Winter Rye in Pots. Length of Stem.*

Planted May 17th.		Planted June 22nd.		Planted July 27th.	
Age (days).	Length (cm.).	Age (days).	Length (cm.).	Age (days).	Length (cm.).
28	0.0	22	0.0	28	0.0
49	1.3	37	0.0	78	0.0
91	69.0	50	1.0	90	0.0
105	63.0	107	31.7		
	flowered		flowered		failed to flower

## EXPERIMENTS OF 1932 AND 1933.

*Experimental Procedure.*

*Cultivation.* The 1931 experiment indicated a close inter-relationship between the temperature after-effect and photoperiodism. It was decided in 1932 to repeat the experiment, using a larger number of replicates and with modifications in the methods of cultivation and of day-length control.

(1) The water-culture method had not given satisfactory vegetative growth in 1931, and it was desirable to introduce manurial variations, thus excluding the possibility of growing in soil. For these reasons, in 1932 the plants were grown in 12-inch unglazed earthenware pots filled with Bedfordshire silver-sand, three plants being grown in each pot. A series of solutions of nutrient salts was made up so that the required quantity could be added weekly to the pots by giving them a mixture of equal volumes of each solution. In this way both 'complete' and 'deficient' solutions could be made up very speedily.

The solution was added through a small pot embedded in the sand, and immediately afterwards each pot received 1 c.c. saturated ferric chloride solution, containing 1 per cent.  $\text{MnSO}_4$ . The nutrients were added weekly during the first eight weeks, and no further additions were made. In all, the fully manured series received the following weights of solid salts (gm. per pot):

Di-sodium hydrogen phosphate	2.385 gm.
Calcium chloride	0.415 "

Magnesium sulphate	1.405 gm.
Sodium nitrate	10.348 „
Potassium sulphate	2.087 „
Ferric chloride	5.800 „
Manganese sulphate	0.0058 „

In 1932 nitrogen and potash deficiency was provided by reducing sodium nitrate to 1/10 in one series, and by entirely omitting potassium sulphate from another. No compensation was made for the diminution of sulphate or sodium, and tap-water was used for the solutions and for watering.<sup>1</sup> It was thought that complete omission of potash would allow such potash as might occur in the pots and sand to provide the small dosage, but apparently the amount present entirely satisfied the requirements of the plants, for there was no sign of starvation and their behaviour was exactly like that of fully manured plants. The potash-deficient series of 1932 are therefore omitted from this account, and the procedure was amended in 1933.

All the series subjected to manurial deficiencies in 1933 were grown in glazed pots, 18 in. high and 6½ in. internal diameter, provided with drainage holes near the base. The old porous earthenware pots were used for fully manured series, but a few plants were grown for comparison in glazed pots with complete nutrients. These grew rather less satisfactorily than those in the old pots, but sufficiently well to show the effects of manurial deficiencies. So that plants could be grown in long and short days under approximately equivalent climatic conditions, an artificial method of shortening the day was adopted. There was evidence in 1931 that a day-length of about 10 hours was critical in the growth of rye, and therefore in 1932 and 1933 the plants under short-day treatment were exposed to daylight only between the hours of 7 a.m. and 5 p.m. (G. M. T.)

For this purpose a double-walled shed was constructed which was light proof, and also heat insulated by a layer of air between the walls. Ventilation was ensured by holes in the floor and a light-proof ventilator inserted in the ply-wood ceiling. The pots under short-day treatment were kept on trucks which were run in or out of the shed on wooden rails covered with iron strips. At planting-out great care was taken that the seedlings destined for short-day treatment never experienced a 'long' day.

To obtain a much higher standard of precision than in 1931, nine replicates were provided in each series for each examination; towards the end of the experiment, when differences were substantial, only six replicates were used. To keep the increased number of series and replicates within the limits of a workable experiment only winter rye was grown, since this

<sup>1</sup> The pH of the solution as added to the pots was 8.5: further trials have shown that a more acid solution (6-7) gives better growth of winter rye.

alone of those plants already studied was an obligatory winter variety, and its young spike was easy to handle and to measure. The scope was further limited by using only two temperatures for germination, namely, 1° C. and 18° C. The seed was graded by inspection and germinated in moist sand; the sowing was planned for planting-out during the first week in May. The treatments used are set out in Table III.

TABLE III.  
*Scope of 1932 Experiment.*

Nutrition.	Temperature at germination.	Length of day.	Abbreviations used in subse- quent tables.
Fully manured	1°	Long (16 hrs.)	FMLD 1°
" "	18°	" "	FMLD 18°
" "	1°	Short (10 hrs.)	FMSD 1°
" "	18°	" "	FMSD 18°
1/10 normal nitrogen	1°	Long (16 hrs.)	N/10 LD 1°
"	18°	" "	N/10 LD 18°

In 1933 more information was required about the early stages of plants which were expected to flower, and in these, examinations were made weekly after the second week from planting.

Table IV gives the scope of the work in 1933. It can be seen that a high degree of potash deficiency was used, viz. (1/100 the amount given to fully manured plants).

TABLE IV.  
*Scope of 1933 Experiment.*

Nutrition.	Temperature at germination.	Length of day.	Abbreviations used in subse- quent tables.
Fully manured	{ 1° and 18°	Long (16 hrs.)	{ FMLD 1°
" "	{ 1° and 18°	Short (10 hrs.)	{ FMLD 18°
" "			{ FMSD 1°
" "			{ FMSD 18°
1/100 normal potash	{ 1° and 18°	Long (16 hrs.)	{ K/100 LD 1°
" "	{ 1° and 18° <sup>1</sup>	Short (10 hrs.)	{ K/100 LD 18°
" "			{ K/100 SD 1°
" "			{ K/100 SD 18°
1/10 normal potash	{ 1° and 18° <sup>1</sup>	Long (16 hrs.)	{ K/10 LD 1°
" "	{ 1° and 18° <sup>1</sup>	Short (10 hrs.)	{ K/10 LD 18°
" "			{ K/10 SD 1°
" "			{ K/10 SD 18°
1/100 normal nitrogen	{ 1° and 18° <sup>1</sup>	Long (16 hrs.)	{ N/100 LD 1°
" "	{ 1° and 18° <sup>1</sup>	Short (10 hrs.)	{ N/100 LD 18°
" "			{ N/100 SD 1°
" "			{ N/100 SD 18°

<sup>1</sup> Provision made for one sample only.

*Sampling.* In 1932 the first sample was taken four weeks after planting, repeated at three fortnightly intervals, and then twice at intervals of three weeks, making six samples in all. Alternating with the early samples, tiller counts were made on a large number of plants. In addition, plants kept in reserve were examined later in the season.

In the sampled plants a complete examination was carried out, and the tillers were counted. Dissection revealed the number of leaves already definitely foliar, and the length and degree of development of the spike. Where elongation had begun, the stem-length was recorded.

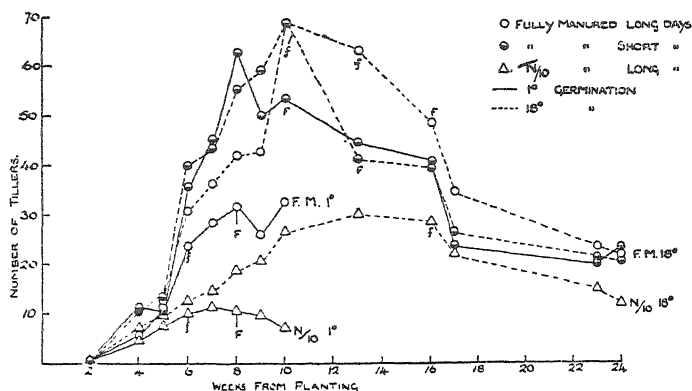
As many investigators have sought an explanation of the mechanism of these flower-promoting stimuli in the varying carbohydrate and nitrogen contents of the plants, and more especially in the relation between these two, it was decided to carry out simple estimations of these quantities. For this purpose one leaf from each plant was used at each sampling date, namely, the youngest fully expanded leaf on the main axis. Such leaves, which will be referred to as the 'dominant leaves' were cut from the plants and prepared for estimations of nitrogen and sugars on the day preceding each anatomical examination. From four of the plants these leaves were dried for nitrogen estimation. They were cut off at the junction of lamina and sheath, quickly carried into the laboratory in a moist vessel, and weighed. The leaves were then dried at 45°–50° C. for an hour, reweighed and stored in tubes sealed with waxed corks. The five leaves for sugar estimation were cut in every case between 2.30 and 3.30 p.m., in order that conditions for assimilation should as far as possible be similar for each sample. Fortunately it was possible to cut each sample in either intermittent or continuous sunshine. The leaves were severed and weighed as those for nitrogen estimations, and immediately cut up into a specimen tube (4 in. x 1 in.), and boiling 95 per cent. alcohol poured over them. This was boiled for a short time and the tube sealed with a waxed cork. Areas were not measured, as rapid killing is essential for the accuracy of sugar analysis. In no case did more than three minutes elapse between cutting and killing.

### *Experimental Results.*

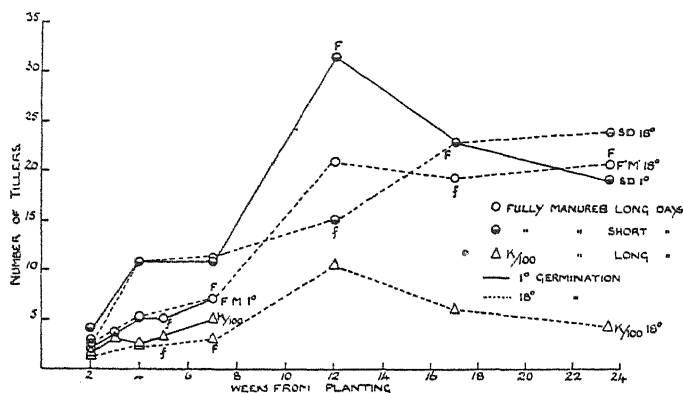
#### *General progress of the plants.*

Text-figs. 4 and 5, which give tiller counts, show that the extent of vegetative growth was widely different in the two years—in 1932 tillering in all comparable series was more than double that in 1933. The poor growth in 1933 was largely due to the abnormally high temperature prevailing, which heated the sand, and in spite of heavy watering produced a wilted condition of the leaves. Another cause was the poor quality of

the seed, which was sprouted and of low germinative power; presumably the still viable seed had also been impaired.



TEXT-FIG. 4. 1932 experiment. Winter rye. Progress of tillering under different conditions of temperature during germination, of day-length and of nutrition. In this figure and in those which follow, 'f' represents the point at which the dissected spikes showed first indication that differentiation was beginning (i.e. by swelling of ridges) and 'F' represents point at which stamen initials could be seen.



TEXT-FIG. 5. 1933 experiment. Winter rye. Progress of tillering under different conditions of temperature during germination, of day-length, and of nutrition.

Despite these strongly marked differences in the vegetative growth, the onset of different stages in the life-history was similar in the two years. This fact gives support to Maximov's (15) view that the control of flowering is not caused by differential vegetative vigour.

Table V gives the number of weeks required to reach various stages in the progress towards flowering.

The effect of germination at 1° C. is very well marked, and also the complete absence of any effect on flowering of nitrogen deficiency. Nitrogen starvation was clearly manifested in a yellowish-green colour and much



reduced tillering; nevertheless flowering was delayed in the 'cold germinated' series by two days only.

TABLE V.  
*1932 Experiment. Progress of Plants.*

	Number of weeks from planting.					
	FMLD 1°	FMLD 18°	N/10 LD 1°	N/10 LD 18°	FMSD 1°	FMSD 18°
Tillering began	2	2	2	2	2	2
'Shooting' began	6	16	<7	16	17	16
Last leaf out	8	17	8	17	23	23
Anthesis	9	20	<10	20	failed	failed
Ripe grain	16	failed	16	failed	,,	,,

In the two series grown in long days after germination at 18°, the ears reached the stage of anthesis in October. In short days the absence of a temperature after-effect may be noted. Ear emergence, and consequently anthesis, did not occur; although there was evidence of shooting after sixteen weeks the stems failed to elongate. A curious feature of the plants grown in short days is the heavy accumulation of waxy bloom on younger leaves of the 'shooting' tillers. This occurs to a smaller extent on all rye leaves during the elongation of the stem. Eventually the ears died within the leaf-sheaths. The death of fully formed ears without emergence was also seen in late sown plants in 1931, and is a characteristic effect of short-day treatment on both summer and winter rye. There is some evidence that it is associated with structural changes in the stem, including phloem necrosis.

In 1933 progress was so similar to that in 1932 that no description is needed. Potash starvation, like nitrogen starvation, had no effect on the date of flowering. The chief difference lay in the behaviour of the 'non-flowering' series. In 1933 signs of shooting did not become apparent until November 7th.

#### *Tillering.*

Tiller counts were made at weekly intervals in 1932, and at each sampling in 1933. The results are shown in Text-figs. 4 and 5 and in Table VI tiller counts are given of those sets which were examined once only after eight weeks.

TABLE VI.  
*Tiller Numbers after Eight Weeks' Growth.*

K/100 SD		K/10 LD		K/10 SD		N/100 LD		N/100 SD	
1°	18°	1°	18°	1°	18°	1°	18°	1°	18°
7.7	10.4	4.3	8.3	7.4	14.7	0.9	1.9	2.0	3.3

Although the difference in actual numbers in the two years is considerable, the relation of these numbers to treatment is similar. In the hot summer of 1933 there were more tillers in all comparable series after two weeks' growth than in 1932, but the rate of increase was far lower; so that the maximum number was less than half that in the cooler year.

(1) *Temperature after-effect.* In 1932, in both the long day series, cold germination reduced tillering rate: but as with flower production, this effect disappeared under short-day treatment. In 1933 the temperature after-effect was less marked. In fully manured plants in either day-length, temperature at germination was without effect on the *rate* of tiller production, although in long days flowering terminated tiller production. With manurial deficiencies a reduced rate of tillering was associated with cold germination.

(2) *Photoperiodic effect.* Short days increased tillering rate, especially in the early stages. In 1932, the tiller number in later stages equalled that of plants germinated at 18°, and grown in long days, but in 1933 the early increase was maintained. This effect is shown with both nitrogen and potash deficiency.

(3) *Effect of manurial deficiency.* In all cases, and in both years, nitrogen deficiency reduced tillering. A small degree of potash deficiency (K/10) has slightly increased tillering in both day-lengths after germination at 18°, but reduced it after cold germination. A greater degree of deficiency (K/100) reduces tillering.

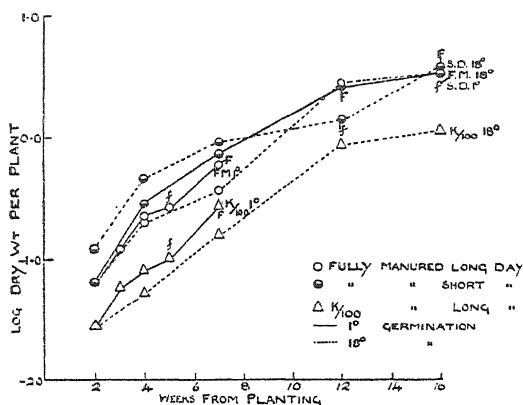
(4) *Relation of tillering to flowering.* The tiller numbers can be grouped according to the flowering behaviour of the plants. Taking results of both experiments into consideration, it is found that at seven weeks from planting, in the six series in which the plants flowered early, the numbers of tillers per plant ranged from 28.4 to 0.9. In the sixteen series in which flowering was delayed, or failed entirely, the tiller numbers ranged from 45.3 to 1.9. Further, where flowering is delayed, the tiller number is on the average higher than when flowering occurs, but it is equally clear that tillering activity has not itself checked the tendency to flower, since flowering has occurred with so high a tiller number as 28.4, and failed when tillers numbered only 1.9. The relatively high tiller numbers resulting from germination at the higher temperature, and from short-day treatment, show no causal relationship with failure or delay in flowering, but may be regarded as an expression of one of the consequences of that failure.

#### *Dry weight.*

In 1933 the whole aerial portions of the plants used were dried and weighed. The logarithms of these weights are shown in Text-fig. 6.

(1) *Temperature after-effect.* Clearly, in long days low temperature germination is followed by a greater rate of dry-weight increase; this

cannot, however, be regarded as the determining factor in flowering since at seven weeks from germination the dry weight was even higher in the



TEXT-FIG. 6. 1933 experiment. Winter rye. Logarithms of dry weight of whole aerial portion of plant.

two 'short-day' series which did not flower. The greater increase in dry weight under short-day conditions is noteworthy.

(2) *Photoperiodic effect.* The very considerable accumulation of dry matter in plants grown in short days continues for seven weeks, after which the rate of increase falls off. This is associated with high levels in both nitrogen and soluble carbohydrates.

#### *Behaviour of apex of main axis.*

THE (N)utrition of floral development has been limited to the main axis; that in tillers which produce an ear, is similar. The measurements made on the main axis were as follows:

(1) Number of leaves already produced at the time of examination. This includes all dead basal leaves, and all young leaves, and excludes ridges whose nature cannot be determined by observation. (2) The length of the spike. (3) The number of ridges. Both (2) and (3) are measured from the last leaf counted under (1), so that (1) plus (3) is the number of lateral appendages the growing point has produced. If from this number is subtracted the final number of leaves found in the series, the remainder gives the number of ridges which will produce flowers. Unfortunately in the series which do not flower, this final number cannot be determined.

(1) *Number of leaves produced on main axis.* The observed numbers are shown in Tables VII and VIII.

In studying leaf production two aspects must be considered (1) rate of differentiation and (2) final level attained before flower differentiation terminates leaf formation.

The *rate* of differentiation is negligibly influenced by germination temperature, short days or manurial deficiency. The final level attained is singularly constant in relation to the flowering habit of the plants. The series failing to flower produced from twenty to twenty-two leaves, those which flowered, ten to thirteen, irrespective of the particular treatment given. It seems as if flowering cannot take place in winter rye germinated at 1° C. until ten to thirteen leaves have been formed, and this is not modified by manurial starvation. If flowering is inhibited a further number of leaves is formed.

TABLE VII.

*Number of Leaves on Main Axis, 1932.*

(The no. in brackets is the number of replicates included where this differs from nine.)

Age in weeks.	FMLD 1°.	FMLD 18°.	N/10 LD 1°.	N/10 LD 18°.	FMSD 1°.	FMSD 18°.
4	11.00	11.23	10.67	11.33	12.33	12.00
6	13.44	13.87	12.25	12.12	14.11	13.89
8	13.33	15.33	12.33	14.33	16.78	16.22
10	13.33	18.25	12.44	17.00	18.11	18.22
13	ears emerged	20.83 (6)	ears emerged	20.00 (5)	19.00 (6)	19.67 (6)
16	—	21.00 (7)	—	20.59 (7)	20.67 (6)	21.25 (8)

TABLE VIII.

*Number of Leaves on Main Axes, 1933.*

Age in weeks.	FMLD 1°.	FMLD 18°.	K/100 LD 1°.	K/100 LD 18°.	FMSD 1°.	FMSD 18°.
2	9.2	9.1	8.7	8.5	—	9.9
3	10.5	—	10.1	—	—	—
4	11.1	10.8	10.1	9.7	—	11.1
5	11.5	—	11.1	—	—	—
7	12.2	12.9	12.0	13.2	13.5	13.6
12	ears emerged	17.7	ears emerged	17.3	18.0	17.4
17	—	22.0	—	—	20.7	20.0

main axes  
destroyed by  
gout-fly

Age in weeks.	K/100 SD 1°.	K/100 SD 18°.	K/10 LD 1°.	K/10 LD 18°.	K/10 SD 1°.
8	12.5 <sup>1</sup>	15.0 <sup>1</sup>	11.6 ears emerged	12.5 <sup>1</sup>	15.2 <sup>1</sup>

Age in weeks.	K/10 SD 18°.	N/100 LD 1°.	N/100 LD 18°.	N/100 SD 1°.	N/100 SD 18°.
8	15.8 <sup>1</sup>	9.8 ears emerged	13.5 <sup>1</sup>	14.2 <sup>1</sup>	14.6 <sup>1</sup>

<sup>1</sup> The full number of leaves had not been formed at eight weeks.

(2) *Number of ridges on spike.* Table IX gives the total number of floral primordia on the undeveloped portion of the apical meristem, as observed in 1932.

It has already been explained that many of the actual ridges present will become leaves. For this reason, the number of ridges observed must be corrected to give an estimate of the true flower expectancy by subtracting the number of ridges destined to become leaves, i.e. the final leaf-number for the series, as measured at a later sampling, minus the leaf-number at the time of the sampling in question. The result of these calculations appears in the Table.

TABLE IX.  
*Number of Flower Ridges. 1932.*

Age in weeks.	FMLD 1°.	FMLD 18°.	N/10 LD 1°.	N/10 LD 18°.	FMSD 1°.	FMSD 18°.
4	4.89	—	5.23	—	—	—
6	28.56	4.74	20.56	0.24	13.67	9.22
8	42.00	13.11	31.14	8.22	26.11	19.90
10	41.45	17.81	34.33	12.25	49.00	36.11
13	—	35.50	—	25.00	55.67	52.50
16	—	31.83	—	34.00	61.67	44.92

This method of correction of ridge number gives some idea of the point in time at which flowering ridges begin to be laid down. In the two low temperature 'long-day' series this is earlier than the fourth week; in the others it is earliest in the two 'short-day' series, and latest with nitrogen starvation (N/10 18° LD.). It is clear that plants grown in 'short' days have a high potential grain-yielding capacity, but actually, as already stated, the spikes die within the leaf-sheaths. By applying long-day treatment at this stage normal ear emergence is induced, and then very large ears with increased grain number are produced.

There is no apparent morphological distinction between ridges which become leaves and those which produce flowers, and it is impossible to say whether in the early stages any actual difference exists, or whether the ridges are at that time plastic structures whose further development is controlled by external conditions.

The constancy of the minimum number of leaves produced suggests, however, that the indeterminancy is true only of the later formed ridges—i.e. after, say, the tenth.

In 1933 these findings were confirmed. Again, the higher proportion of floral ridges in short-day plants appeared. The effect of low temperature germination and of short days was exactly the same as in 1932. Potash starvation in 'long-day', cold-germinated plants was without any effect on

ridge production, but with warm germination, had a depressing effect which became more marked as time went on.

(3) *Length of 'spike'.* 'Spike' length (i.e. the length of the undifferentiated portion of the apical meristem) cannot be regarded as an absolute criterion of reproductive activity. Its rate of increase does, however, bear a close relationship to the onset of flower differentiation.

TABLE X.  
*Spike-length (mm.), 1932.*

Age in weeks.	FMLD 1°.	FMLD 18°.	N/10 LD 1°.	N/10 LD 18°.	FMSD 1°.	FMSD 18°.
4	0.61	0.55	0.58	0.52	0.86	0.56
6	2.66	0.82	1.60	0.71	1.25	1.22
8	33.43	1.18	44.21	0.87	1.85	1.45
10	116.67	1.49	84.33	1.12	3.89	2.50
13	—	2.24 (6)	—	1.80 (6)	5.00 (6)	4.13 (6)
16	—	3.38 (6) <sup>1</sup>	—	2.39 (6) <sup>1</sup>	7.86 (6)	4.99 (6) <sup>1</sup>
		16.46 (7)		16.32 (7)		11.28 (7)

In Table X is shown the progress of spike-length in the 1932 experiment, and Text-fig. 7 gives the same data expressed on a logarithmic scale. The graph shows clearly the slow exponential growth during the first thirteen weeks in the four series in which flowering was delayed. At the sixteenth week, in three series more rapid increase in length was found to have occurred, but this was due in each case to one plant in which flower development had begun. The exclusion of these plants gave mean values which fell on the same logarithmic curve as those obtained from earlier samples. Morphological evidence of flower initiation was visible in the two 'short-day' series at the tenth week, and in the two long-day series germinated at 18° C. such evidence appeared after thirteen weeks.

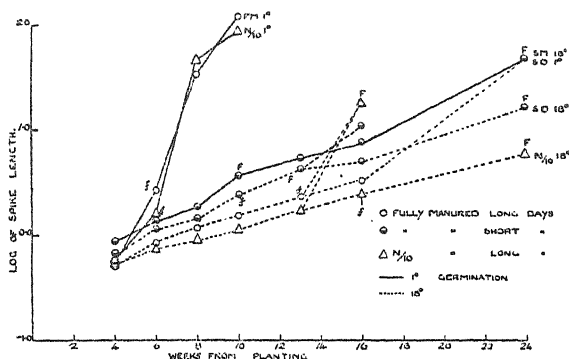
A few remaining plants of the four series which had not flowered were examined in October. The ears were then ready for emergence, and had attained their full length. Again the logarithms of the mean spike-length fell on the same straight line for each series. Thus a length and differentiation fit for flowering had been attained by slow exponential growth over a long period of time (six months), and in these spikes there had been no 'grand period of growth'.

In the two series grown in long days after germination at 1° C. the spikes grew for about four weeks at the same slow rate as those which did not flower. After this, however, growth and differentiation were very

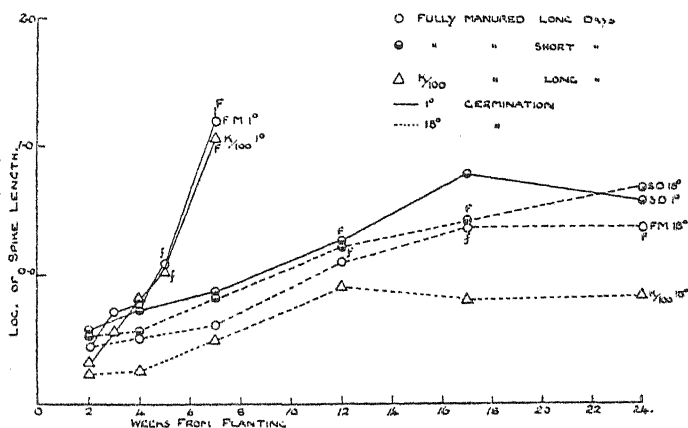
<sup>1</sup> Where two values are given the higher value includes a single abnormally long spike, while in the lower values of the mean this has been omitted.

rapid, and the ears were ready to flower by the tenth week. Nitrogen deficiency was virtually without effect on the rate of growth of the ear.

Despite the weaker vegetative growth in 1933 the behaviour of the



TEXT-FIG. 7. 1932 experiment. Winter rye. Logarithms of spike-length.



TEXT-FIG. 8. 1933 experiment. Winter rye. Logarithms of spike-length.

spike initials resembled that observed in the earlier experiment (Text-fig. 8). Germination at 1° C. produced plants which when grown in long days, eared in about ten weeks; a high degree of potash starvation had no effect in extending this period or in impairing the ears.

In short days, or in long days after germination at 18° C., slow exponential growth in length took place, but at a lower rate than in the previous year, differentiation also was relatively delayed. Potash starvation had the effect of still further delaying differentiation in the 'long-day' series after germination at 18° C., with the result that up to the twenty-third week, only one plant showed signs of differentiation. The rate of growth in length was greatly retarded by deficiency of potash.

In both years it was clear that short days promoted both growth in length and differentiation to a greater extent than long days after germination at 18° C.

In 1933 a high degree of nitrogen deficiency was used, both in long and short days. In long days this had little effect on spike-length, and in short days retarded growth of the spike. Maximov (15) and Borodin (4), however, found that in barley nitrogen deficiency removed the inhibiting effect of short days—no indication of this effect has been observed with either winter or spring rye.<sup>1</sup>

#### *Short day after-effect.*

After three and a half weeks' growth, a pot among the SD. 1° plants was slightly damaged, and to avoid inclusion in samples was removed and thenceforward exposed to the full length of day. Eight weeks later, this was noticed to be in ear—the low temperature germination effect which had been masked by short days, manifested itself in exactly the number of weeks required by L.D. 1° plants to reach that stage from planting. The ear was very long—due to the large number of ridges already laid down during its exposure to short days. Later, a pot of SD. 1° and one SD. 18° were given long days after thirteen and a half weeks 'short-day' treatment. Four weeks later in the '1° C.' pot, three plants were coming into ear; meanwhile those germinated at 18° C. resembled the control 'short-day' plants, at least externally. Eventually during October, after about twelve weeks of LD. treatment two of the three plants formed ears, differing in this respect from both LD. 18° and SD. 18° plants. The rapidly shortening days stopped ear-emergence, but all the floral parts were completed. It will be remembered that in 1931, 18° plants grown through the winter produced ears in the lengthening days of March: *it seems that short days promote the inception of flowering* but prevent its completion, which can only occur in long days. This would account for the relatively long spikes met in both years in SD. plants.

#### V. CARBOHYDRATE AND NITROGEN CONTENT.

The importance of the relations of carbohydrate to nitrogen content as a causal agent in flower production has been stressed by some investigators. Thus, Klebs (12), Kraus, and Kraybill (13) both support this view. Gassner (8) also found higher sugar content in winter cereal seedlings when these were germinated at 1° C. than after germination at higher temperatures, and relates this fact to their flowering after spring sowing. On the other hand, the importance of this relation has been denied by others, among whom may be cited Arthur, Guthrie and Newell (3).

<sup>1</sup> The result with spring rye was obtained in the present year (1934).



To investigate this point, estimations of organic nitrogen and soluble carbohydrates were made, using the youngest expanded leaf on the main axis, prepared as described on p. 931. The total organic nitrogen, the total soluble carbohydrate and reducing sugar were alone estimated, as this was considered sufficient to determine any large effects the various treatments might have had on the plants' metabolism.

*Methods of analysis.*

(1) *Nitrogen.* In this work the total nitrogen only was estimated, using the Pregl micro-Kjeldahl technique (20). No special precautions were taken to estimate nitrate present.

To reduce sampling error the dried leaf material was finely ground in a Wiley mill: from this powder, after redrying duplicate samples of from 10-18 mgm. were weighed out on a micro-balance. The variation between the nitrogen in duplicate samples was between 2 and 3 per cent. of the mean.

(2) *Sugars.* The material had been stored as described on p. 931 in the 95 per cent. alcohol in which it had been killed. This was dealt with by a modification of the method described by Archbold (2), and reducing and total sugars were estimated by the ferricyanide method of Hagedorn and Jenson.

*Experimental results.*

(1) *Nitrogen.* The percentage of total nitrogen in the dried leaf material at the sampling dates is shown in Text-fig. 9.

It is clear that flowering occurred under widely different conditions of nitrogen content, while similar nitrogen content (as in the two nitrogen starved series N/10 1° and N/10 18°) may be associated with either early or late flowering. Short-day treatment increased the nitrogen content during the first ten weeks.

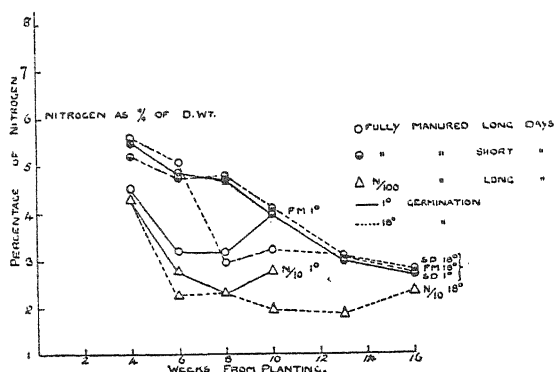
(2) *Reducing sugars.* In Text-fig. 10 is given the concentration of reducing sugars at different ages, expressed as milligrams of glucose per gram of fresh leaf.

Little variation is shown, but a marked accumulation occurs after eight weeks in the two sets which were then about to flower. Differentiation of flower primordia had taken place four weeks earlier and therefore cannot be regarded as a consequence of this accumulation. More probably, the leaves on the expanding stem intercepted a small proportion of the flow of carbohydrate to the growing ear.

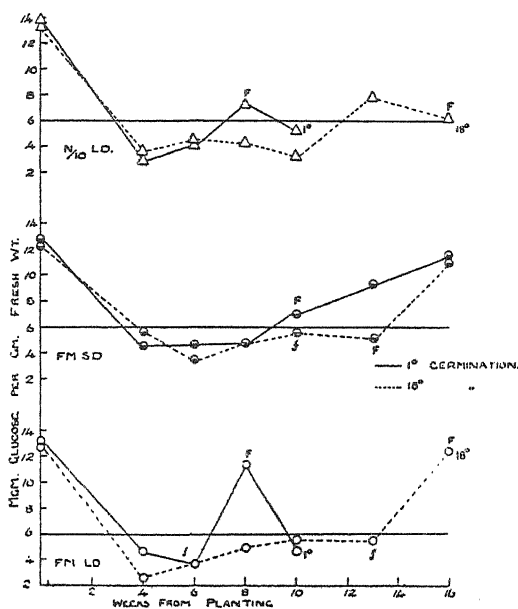
Manurial treatments had no effect on reducing sugar content.

(3) *Total sugars.* The results are shown in Text-fig. 11. At the time of planting the sugar content was not substantially affected by temperature during germination, and at no stage was a temperature after-effect

uniformly apparent. The factor which dominates sugar content is nitrogen starvation which substantially increases it both where flowering occurs and



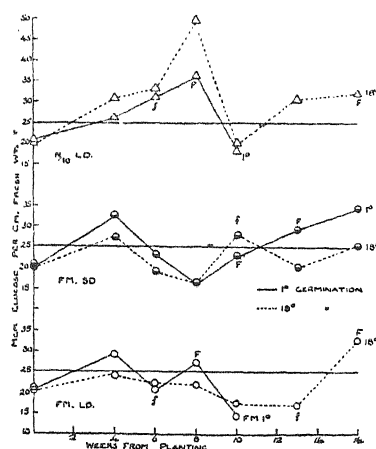
TEXT-FIG. 9. 1932 experiment. Winter rye. Nitrogen content of 'dominant' leaf expressed as percentage of dry weight.



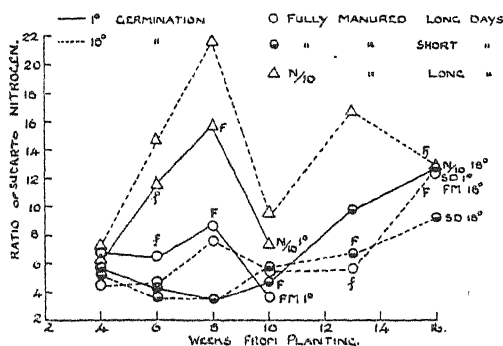
TEXT-FIG. 10. 1932 experiment. Winter rye. Reducing sugar in 'dominant' leaf, expressed as milligrams of glucose per gram fresh weight.

where it fails. Short days which reduce the daily assimilating period by nearly 40 per cent. have a negligible effect on sugar content, a fact which is in complete agreement with the dry-weight data on p. 934. Some explanation of the effect of short days on flowering must be sought which does not postulate a reduced carbohydrate supply. This accumulation of carbohydrates does not result from a low nitrogen content (p. 941).

(4) *Carbohydrate-nitrogen ratio.* Text-fig. 12 gives the ratio of total sugars to nitrogen content. This emphasizes the fact that in winter rye,



TEXT-FIG. 11. 1932 experiment. Winter rye. Total sugars in dominant leaf, expressed as equivalent milligrams of glucose per gram fresh weight.



TEXT-FIG. 12. 1932 experiment. Winter rye. Ratio of total sugars to nitrogen.

this ratio has no relationship with the ability of the plant to differentiate flower initials or to produce flowers. In all the graphs the time of first inception of flowering (*f*) and advanced differentiation (*F*) are marked. In some cases the first stage occurred between samples and was thus missed.

## VI. DISCUSSION.

### *General considerations.*

The problems with which the present investigation is concerned are posited in the introductory section. The approach to the solution lies through a consideration of the 'failure to flower', rather than in the analysis of the causal factors determining flowering.

In all previous work dealing with the particular problem under investigation little information is to hand as to the actual condition of the meristematic regions, or the relation of flowering and its failure to the vegetative growth of the plants treated. There was some reason to suppose, *a priori*, that the failure to flower in certain of the treatments may have been due to a direct antagonism between tendencies to vegetate or reproduce. This seems to be the position postulated by Maximov. Thus lack of potassium leads to failure to flower in barley, and in this case the phenomenon is associated with a tendency towards excessive tiller formation. Two or three successive cycles of tiller formation occur, each associated with the death of older tillers at the time when the ear should normally begin its phase of rapid development (14). A fairly complete study of the morphological development was therefore undertaken to inquire into the importance of this aspect. The results (Text-figs. 4, 5, and 6) show conclusively that the extent of vegetative development is a consequence of flowering and in no way exerts a causal influence. Thus under nitrogen starvation flower primordia appear and flowering takes place at the normal time in spite of reduced vegetative growth, whereas the temperature after-effect is not influenced by nitrogen starvation. Conversely the photoperiodic effect also is little affected by nitrogen starvation; under short days there is an increase in tiller numbers both with ample and deficient nitrogen supply, and a slight reduction in differentiation of the spike was noted as compared with the fully manured plants under these conditions. The findings of Maximov in this respect have not been confirmed; suppression of the vegetative tendency leaves flowering unaffected.

In furtherance of this mode of approach chemical analyses of the plants with respect to carbohydrate and nitrogen metabolism were undertaken. Again the results (pp. 941-3) show that neither carbohydrate level, nor nitrogen content, nor their ratio, has any causal relation to failure to flower. This conclusion was apparent in the course of the work. A rapid survey of the phenomenon of failure to flower in other plants was therefore undertaken collaterally with this investigation. Variation in the length of day provides a ready method for controlling the incidence of flowering, and was used in this preliminary survey.

In relation to the problem of failure to flower the morphological structure of the plant is not without significance.

The distribution of the flowers in relation to the vegetative organs gives a basis on which plants may be divided into two classes:

- (1) Forms with indefinite apical growth and axillary flowers.
- (2) Forms in which the basal region of the axis produces leaves with vegetative axillary buds, and the upper region, a terminal inflorescence with bracts, which may in some cases be suppressed.

In the first type flowers may potentially be produced in the axil of

any leaf. If flowers fail to appear in the appropriate position the question arises as to the mode of failure. Three types of behaviour have already been distinguished: (a) buds develop as long vegetative shoots (*Ipomaea* in long days); (b) flower buds are first produced, and later shed by formation of an absciss layer (*Phaseolus multiflorus* in short days)<sup>1</sup>; (c) no external signs of any bud formation can be seen (*Convolvulus tricolor* in short days).

It is evident that here there is no morphological necessity for vegetative growth to stop when flowering begins, and in many cases the two phases continue together. In others, however, growth of the terminal bud ceases when flowering begins and as a consequence of flowering.

In the second type some vegetative growth must precede the formation of the inflorescence. The extent of this growth will depend on whether a determinate number of leaves is necessary or not. The cereals in general belong to this type, and all the plants studied may be placed in this category. There is evidence to show that the spring and winter cereals differ in this respect, also a difference between species is evident.

The information gained from the present investigation on this point may be summarized as follows.

(1) No known treatment can induce differentiation of flower initials before a certain minimal number of leaves has been formed. Under long days in spring varieties, this number is approximately seven, in winter rye germinated at 1° C., it is about twelve, and in winter rye germinated at 18° C. it is from twenty-two to twenty-five.

(2) In days of ten hours duration the number of leaves produced before differentiation of flowers is always about twenty-two, irrespective of variety or temperature treatment.

#### *Temperature after-effect.*

It appears therefore that the effect of temperature of germination on the time of the production of flower primordia is linked up with the length of the vegetative phase controlled by the obligatory minimal number of leaves which must precede flower formation. This is the major after-effect of temperature.

In addition, however, low temperature germination directly affects the

<sup>1</sup> *Phaseolus multiflorus* has been variously described as a 'long-day' plant (Maximov 17) and as a 'short-day' plant Tincker (24). An experiment performed on this plant by the author has thrown light on this question. Under conditions of short days, flower-bud production began earlier than with long days, and was associated with reduced vegetative growth and a considerable weakening of the twining main axis. Flower inception had thus begun in short days, while under long-day conditions buds similarly situated were growing vegetatively. Under continued short-day treatment these flower buds were shed following the production of a very definite absciss layer. These flowers were quite normal—one such flower, which for some reason escaped abscission produced an unusually large fruit cf. Tincker (24). Control plants under the natural summer day behaved normally. In this connexion it is interesting to note that Garner and Allard (6) found that short days were in many cases essential for normal abscission of leaves in autumn.

rate of elongation of the apical meristem (see Text-figs. 7 and 8). When the plants germinated at  $1^{\circ}\text{C}$ . and exposed to long days are compared with plants similarly treated but germinated at  $18^{\circ}\text{C}$ . it is seen that from the second week onward there is a much more rapid elongation of the spike in the former, and this antedates the appearance of flower primordia. The requisite number of leaf primordia is reduced by the  $1^{\circ}\text{C}$ . treatment, and in addition the rate of growth of the spike is increased, hence there is a twofold acceleration of flower primordia initiation.

Gassner (8) found that the length of time taken to flower increased with increasing temperature at germination—it may then be suggested as a hypothesis that as the temperature at germination approaches a minimum, the minimal number of leaves approaches that found in the summer variety. On the other hand, the close relationship observed in Petkus rye between minimal leaf numbers for the summer variety—seven, for ‘cold’ germination in the winter variety—twelve, and for warm germination in the latter—twenty-two, suggests a possibility that leaf formation may occur in cycles of seven.

#### *Photoperiodic effect.*

*Short days* delay the production of flower primordia by raising uniformly to approximately twenty-two the minimal number of leaves required. This effect is unaffected by the temperature of germination. A further effect of short days is seen in the rate of production of floral ridges on the spike (Table IX). At ten weeks the average numbers were as follows: FM SD.  $18^{\circ}$ , 36.1; FM LD.  $18^{\circ}$ , 17.8. Meanwhile the FM. SD.  $1^{\circ}$  plants showed 49.0. The accelerating effect of low temperature germination is thus still evident. Further, these figures account for the earlier differentiation in short days after high temperature germination. The result of this earlier differentiation is apparent in the experiment described on p. 940. By removing plants after thirteen and a half weeks, from short days to long days, ear emergence occurred after a further twelve weeks in the  $18^{\circ}\text{C}$ . plants, whereas similar plants which had been exposed all the time to long days had not yet eared. Plants which had been germinated at  $1^{\circ}\text{C}$ . and removed from short days at the same time required only four weeks of long-day treatment for ear emergence.

Short days therefore lead to earlier differentiation of flower primordia by increasing the rate of growth of the meristem, and this partially counteracts the effect of increasing the minimal number of leaves: low temperature germination still further counteracts this effect by inducing a still more rapid growth of the meristem.

One of the effects of this extension of the meristem in short days is made manifest by subsequent removal to long days, when the extra ridges

differentiated give rise to abnormally long ears, with more than the normal number of grains (p. 940).

Since the above was written, the author has found that N. A. Maximov (16) considers that short days exert an accelerating effect on flowering; this appears in an abstract of a paper presented at the Fifth International Botanical Congress, though not read. He suggests that the physiological characteristic of winter crops is the existence of a factor antagonistic to flowering, and that the action of this factor can be checked by long exposure to cold, by short days, or by X-ray treatment.

### *Stages of flowering.*

So far the conditions leading to the formation of flower primordia have alone been considered. The whole process of flowering may be divided into the following phases:

- (1) Attainment of 'ripeness to flower' (Klebs).
- (2) Differentiation of flower primordia (flower initiation).
- (3) Further development of the flowers up to ear emergence or anthesis.

The stage of 'ripeness to flower' begins with the production of the minimal leaf number (itself determined by temperature of germination and day-length). That this is so is shown by two considerations:

(1) In short days flower primordia continue to differentiate indefinitely, in spite of the fact that further development of those formed earlier is delayed.

(2) Flower differentiation begins, not just above the last formed leaf primordia, but at the middle of the spike.

In this connexion the photoperiodic after-effects noted by Rasumov are important. Analysis of his figures for short-day plants (millet) discloses the fact that no photoperiodic after-effect can be induced until three days from germination have elapsed. After this the effect of the day-length can be completely accounted for on the assumption that the rate of primordium differentiation is increased by short days. The evidence on which such an assumption is based is given in Table XI, taken from Rasumov's data (21).

When short days are given first, it can be seen that three preliminary short days have no effect on the time taken to flower—four days' short-day exposure, however, shorten the period by four days, and six days' exposure shorten the time by twenty-five days. Further extension of the preliminary 'short-day' period to nine days has little effect. We may therefore suppose that six days of short-day treatment are required in millet to attain the condition 'ripeness to flower', and about twenty further days of time, *irrespective of day-length*, to produce an emerging panicle.

TABLE XI.

*Photoperiodic After-effect. Millet (from Rasumov).*

Treatment.	Time for ear emergence (days).	Long days.	Short days.	State of ear at change of day-length.
All long days	51	51	0	no record
22 long days at start	42	22	20	"
15 " "	38	15	23	"
10 " "	31	10	21	"
6 " "	27	6	21	"
All short days	23	0	23	"
3 short days at start	51	48	3	not differentiated
4 " "	47	43	4	"
5 " "	33	28	5	"
6 " "	26	20	6	"
7 " "	28	21	7	"
9 " "	24	15	9	"

In long days, the attainment of ripeness to flower is much slower; since with long-day treatment only, fifty-one days are required for flowering, and since we suppose twenty-one days are needed to convert the condition of 'ripeness' into flowering, then thirty long days are needed to attain this condition. Thus *differentiation is five times as rapid under short- as under long-day treatment.*

Since differentiation is so slow under long-day treatment when applied first, the length of exposure up to thirty days does not very much affect the number of subsequent short days required.

TABLE XII.

*Number of Short Days Equivalent to Preliminary Long Days. Millet.*

(30 long days are equivalent to 6 short days).

Preliminary long days.	Equivalent short days.	Subsequent short days.	Calculated number of short days required for earing.
22	4.4	20	24.4
15	3.0	23	26.0
10	2.0	21	23.0
6	1.2	21	22.2
0	0.0	23	23.0

It is apparent from Table XII that millet requires approximately twenty-three short days for earing to take place. If long days are given first the time to ear is increased, since differentiation takes place at only one-fifth the rate. When allowance is made for this the calculated equivalent number of short days required is always approximately twenty-three, whatever the previous treatment.

The figures given by Rasumov for a long-day plant are shown in Table XIII.



TABLE XIII.

*Photoperiodic After-effect. Barley (from Rasumov).*

Treatment.	Time for ear emergence (days).	Short days.	Long days.	State of spike at change of day-length.
All short days	87	87	0	
25 short days at start	68	25	43	differentiated
20 " "	64	20	44	" "
15 " "	59	15	44	not differentiated
10 " "	56	10	46	" "
All long days	50	0	50	
7 long days at start	84	77	7	not differentiated
10 " "	68	58	10	" "
15 " "	58	43	15	ear initial "
20 " "	56	36	20	" "

It is clear that the case of barley is different from that of millet, apart from the obvious difference in its reaction to day-length. In millet it was seen that when once the condition of ripeness to flower is established, the further growth rate is unaffected by day-length. In barley, and also in oats, it will be shown that after differentiation is initiated long-day treatment shortens the time taken to flower: it is therefore obvious that long days are here favourable to the later stages of flower development.

In the cases where short-day treatment is given first, it appears that however extensive the 'short-day' period may be, up to twenty-five days, approximately forty-four long days are needed to complete the process of flowering up to ear emergence. In order to gain further information from these figures, it is necessary to determine the number of long and short days respectively needed to attain 'ripeness to flower'. Rasumov's morphological observations show that for differentiation to be apparent, between ten and fifteen long days are needed, or between fifteen and twenty short days. It seems then that this initial stage is here less dependent on day-length than it is in a short-day plant.

It is possible to assess the relative value of long and short days in promoting the whole process of flowering, as shown in Table XIV.

TABLE XIV.

*Relative Flower-promoting Values of Short and Long Days. Barley.*

Total number of long days to flower = 50.			
" " short " " = 87.			
Long days at start.	Remaining long days.	Equivalent short days (from Table 13).	SD/LD.
0	50 (50-0)	87	1.74
7	43 (50-7)	77	1.79
10	40 (50-10)	58	1.45
15	35 (50-15)	43	1.23
20	30 (50-20)	36	1.20

It can be seen that a preliminary period of long-day treatment of seven days requires subsequently either forty-three long days or seventy-seven short days, that is to say, the flower-promoting value of one long day is equal to that of 1.8 short days. When no preliminary long days are given, the same ratio holds, so presumably during seven long days nothing has been contributed to the process of flowering, or, in other words, 'ripeness to flower' has not been attained. But after ten preliminary long days the subsequent short-day period required is relatively reduced, which indicates that during the ten days some part of the development following 'ripeness to flower' has already taken place. It is probable then that between seven and ten long days are required to attain this condition—a conclusion which agrees with Rasumov's statement that morphological differentiation becomes apparent between ten and fifteen days.

When short-day treatment is applied first, it is seen that ten short days require only three subsequent long days more than are required by twenty-five preliminary short days. This indicates that ripeness to flower has been attained in ten short days, and that the further development is so slow in short days that fifteen further short days have little effect, and therefore after twenty-five short days forty-three long days are still required.

There is evidence for a real 'after-effect' only in the case of the short-day plants. In the long-day plants, the effect is due entirely to the fact that long days hasten the events after flower initiation has been reached. In all respects the findings of Rasumov agree with the results obtained in this investigation.

The conclusions derived from re-examination of Rasumov's data may be summarized as follows:

- (1) In a 'short-day' cereal, like millet, short days hasten differentiation of flowers, and the subsequent stage of development, leading from differentiation to flowering, is independent of day-length.
- (2) In a 'long-day' cereal, like oats or barley, differentiation is almost independent of day-length or may be accelerated by long days, but later stages are considerably hastened by long-day treatment.

If the differentiation of flower primordia is to be accepted as the basis of classification of long- and short-day plants, it will be seen that the spring varieties are all 'long-day' plants, flower differentiation being independent of day-length. The time of initiation, however, as stated above, is dependent on day-length owing to the effect on the minimal leaf number, i.e. onset of ripeness to flower. Winter rye, on the other hand, when germinated at 1° C. is a long-day plant, while germinated at 18° C. it behaves like a short-day plant in that differentiation proceeds more rapidly in short days.

*Further development of the primordia.*

The further development of the flower primordia is unaffected by temperature of germination; day-length, however, is a controlling factor.

Text-figs. 7 and 8 show that the further growth of the spike after flower initiation may follow one of two courses:

(1) either the slow exponential growth prior to flower differentiation continues unchanged (short days);

(2) a sudden increase in growth rate occurs (long days).

Even under conditions of slow exponential growth in continued short days the ear may reach complete development, attaining its normal or a supernormal length (p. 938); normal emergence, however, does not occur, and instead the ear dies within the leaf-sheaths. This death is associated with failure on the part of the stem to elongate. Evidence is to hand that normal development of the vascular supply also fails. Phloem necrosis has been observed, and this may be the proximal cause of the failure of elongation and emergence of the ear. The stem has apparently specific photoperiodic requirements (Wanser (25)).

The cereal plant would appear to occupy a category of its own in relation to photoperiodism. The spring varieties are true long-day plants. The winter varieties germinated at high temperatures are short-day plants with respect to differentiation of flowers, but demand long days for subsequent stages; the winter varieties germinated at low temperatures are similar to spring varieties.

The relation of these varieties to the time of sowing is now clear. Germinated in the early winter at high temperature or at low, flowering takes place simultaneously with lengthening days in the spring. The short days of the winter have in both cases raised the minimal leaf number, and growth in the short days is so slow that the stage of ripeness to flower is not reached until the days are lengthening in the spring. Further development is thus the same in both. Sown in spring the date is of importance, as is also the temperature of germination. At high temperatures the minimal number of leaves is increased, and therefore the phase of 'ripeness to flower' is not reached until day-length is reduced below the limit for elongation and ear emergence. Low temperature is now effective, since by reducing the minimal leaf number the phase of ripeness to flower is attained earlier while long days still prevail. The earlier experiments of Gassner (8) thus find here their logical exposition.

*Internal factors leading to flowering.*

The causal mechanism by means of which the external factors here discussed bring about changes which lead to flowering is little understood. It seems probable that a chemical mechanism is concerned, but the present

investigation has shown no relationship between flowering phenomena and the variations in carbohydrate and nitrogen content. Possibly some substance in the nature of a hormone may be shown to play a part, and work on this line is now proceeding.

### *Vernalization.*

A practical application of the interaction between temperature at germination, length of day, and time of flowering, is seen in the process of vernalization. This term is suggested by White and Hudson (26) as the English equivalent of the Russian 'Jarovizatzia'.

For a complete understanding of the process of vernalization studies of primordium differentiation seem to be essential. The findings in this work are essentially in agreement with the Russian work, save only Lyssenko's suggestion as to the efficacy of continuous darkness in the early stages. This is now receiving further investigation.

## VII. SUMMARY AND CONCLUSIONS.

(1) The reaction of winter rye (var. Petkus) to temperature at germination and to varied day-length has been investigated. Germination at temperatures a little above freezing-point substantially hastens the flowering of plants sown in spring. When, however, the plants are exposed to the short days of winter or to a day-length artificially shortened to ten hours, this hastening effect is not manifested.

(2) Investigation of early stages in flower formation show that after germination of  $1^{\circ}\text{C}$ ., artificially shortened days retard flower differentiation. After germination at  $18^{\circ}\text{C}$ ., however, short days hasten it to a small extent, although in this case long days are necessary to complete the process of flowering.

(3) Experiments with spring rye (var. Petkus) show that temperature at germination has no effect on the rate of flower production, which is even more rapid than that of the winter rye germinated at  $1^{\circ}$ . Short days inhibit flowering, and it may be said that summer rye, and winter rye germinated at  $1^{\circ}$ , react in the same way to length of day as do typical long-day plants.

(4) Reduction of nitrogen supply had no effect on flowering under any day-length, but reduced vegetative growth.

(5) Reduction of potash supply had no effect on flowering after germination at  $1^{\circ}\text{C}$ . but appeared slightly to delay flower differentiation after germination at  $18^{\circ}\text{C}$ .

(6) An estimation was made of the concentration of sugars and of nitrogen in leaves of the different series of plants. There is, however, no evidence that such differences as were observed bear a causal relationship

to flowering. An increase in reducing sugars was apparent just before flower emergence, but since this was preceded by flower differentiation it may be regarded as a result rather than as a cause of the onset of the reproductive phase.

(7) A consideration of these results leads to the following conclusions.

(a) In assigning a plant to its photoperiodic category the *time of formation of flower primordia* should be considered rather than the time of emergence of the inflorescence.

(b) In winter rye, the differentiation of flower primordia is subject to an interaction between day-length and the temperature during germination, which factors determine both the minimal number of leaves formed before differentiation of flower primordia begins, and the rate of growth of the meristematic tissue.

(c) In consequence of this interaction, in long days low temperature germination hastens flower inception in winter rye, whereas in short days this effect is not manifested. At the same time, after germination at high temperatures, short-day treatment leads to earlier differentiation of flower primordia than does growth in long days.

(d) In spring rye there is no 'temperature after-effect' but short days retard the differentiation of flower primordia.

(e) When this differentiation has begun, further development is always hastened by long days. Therefore, if ear emergence is the criterion of flowering, rye is in all cases a long-day plant, but when differentiation of flower primordia is considered, it can be said that winter rye after germination at high temperature is a 'short-day' plant.

This investigation was undertaken at the suggestion of Dr. F. G. Gregory. My thanks are due to Professor V. H. Blackman and to Dr. Gregory for their stimulating criticism and advice throughout the progress of the work, and especially to the latter for help with the discussion section of the paper.

The experimentation (apart from chemical analyses) was carried out at Chelsea Physic Garden, and I wish here to tender thanks to Mr. William Hales, the Curator, for his unfailing helpfulness, and in particular for providing for the daily transport of plants subjected to 'short-day' treatment. My thanks are due also to Mr. H. Tooley for taking the photographs.

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EXPLANATION OF PLATE XX.

Illustrating Miss Olive Norah Purvis' paper on 'An Analysis of the Influence of Temperature during Germination on the subsequent Development of certain Winter Cereals, and its Relation to the Effect of Length of Day'.

Fig. 1. The shed and trolleys used for artificially shortening the day-length.

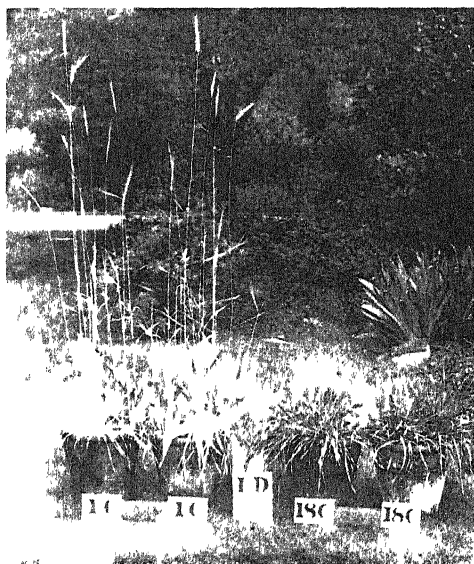
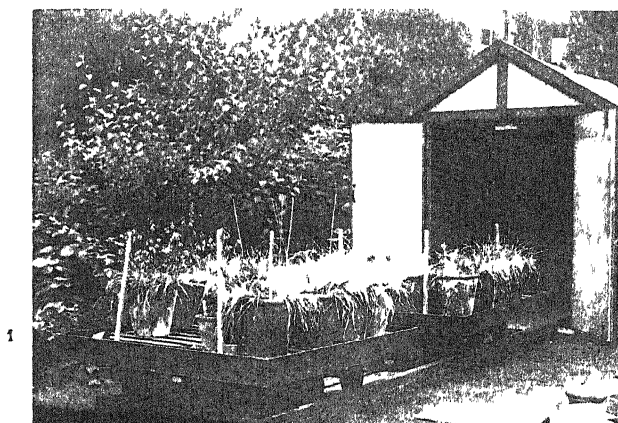
Fig. 2. 1932 experiment. Winter rye growing in long days, and complete nutrients. A temperature after-effect is observed. Photographed eleven weeks after planting.

Fig. 3. 1932 experiment. Winter rye growing in long-days and nutrients deficient in nitrogen. A temperature after-effect is observed. Photographed eleven weeks after planting.

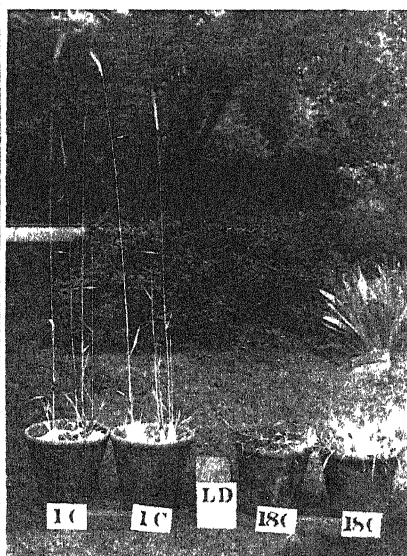
Fig. 4. 1932 experiment. Winter rye growing in short days, and complete nutrients. No temperature after-effect is observed. Photographed eleven weeks after planting.







2



3





## Chemical Studies in the Physiology of Apples.

### XV. The Relation of Carbon Dioxide Output to the Loss of Sugar and Acid in Bramley's Seedling Apples during Storage.

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SOME preliminary experiments carried out by Lall (3) in this laboratory, on the relation between carbon dioxide output and the loss of sugar and acid in the apple fruit during storage, suggested that in the Bramley's Seedling variety the carbon lost as carbon dioxide was insufficient to account for all that lost by the loss of sugar and acid, while no such difference was found in the Worcester Pearmain variety. The measurement of sugar and acid losses presented some difficulty as the rate of loss is very small and the material extremely variable in sugar content. Estimates of such losses made by the usual method of comparing sugar and acid contents of samples drawn at random at suitable time intervals from a population of apples are not sufficiently accurate owing to the practical difficulties involved in dealing with the large samples necessary. To overcome this difficulty Lall (3) employed a method in which half of each apple in a sample is employed for chemical analysis, the other halves being first used for determination of carbon dioxide output under carefully controlled conditions and subsequently analysed. Complete data were obtained for only seven apples for each variety, a number of results being lost owing to the various experimental difficulties encountered during the work. Consequently Lall regarded his results as tentative, and suggested the need for further improvement in the method employed. The present paper deals with attempts to improve the 'half-apple' method and a repetition of the experiments with Bramley's Seedling.

*Distribution of sugar in the apple.*

Lall cut his apples through calyx and stalk and as far as possible to give equal distribution of the area of deeper skin colour, i.e. the blushed side. He found that the difference in sugar content between two halves of an apple was considerably less than that between separate apples. Some estimates of the sugar content of different parts of the fruit were made by the late Dr. D. Haynes (2) with the object of determining whether this method of cutting gave the most symmetrical distribution with respect to sugar and therefore the minimum difference between the sugar content of two halves of an apple. Dr. Haynes' results are shown below in Tables I and II. The dry weights (an approximate measure of sugar content) of portions of the pulp from the calyx and stalk ends of the apple, of inner and outer portions of the pulp and of plugs of tissue removed from the 'blushed' and 'unblushed' sides and intermediately were determined. From the tables it may be concluded that in general sugar concentration increases from the stalk end towards the calyx, from the inside towards the outside, and is lower on the unblushed than on the blushed side of the fruit. With such a distribution of sugar concentration it is clear that the least difference in sugar content between the two halves of an apple will be obtained by adopting Lall's method.

Owing to the uneven distribution or absence of red or yellow skin colour in many cases, and to lack of symmetry in shape it is found impossible in practice to determine with any certainty the areas of maximum and minimum sugar concentrations by relying only on colour distribution on the skin. It is, however, evident that if the fruit were cut in two planes through the calyx and stalk at right angles to one another, wherever the cuts are made the diagonally opposite quarters taken in pairs should have a more nearly equal distribution of the parts of higher and lower sugar content than two halves obtained by cutting the apple once. By this means a small difference of sugar content between two halves should be obtained without dependence on the distribution of skin colour.

Preliminary experiments showed that the increased risk of accidental fungal infection in cutting the apple in quarters instead of in halves was negligible and, further, the larger cut surface exposed did not prolong unduly the period of increased respiration rate due to injury.

Accordingly the sugar and acid contents of each of the four quarters of nine apples from the population used in the experiments described later (pp. 962-6) were determined, so that the differences between the concentrations of these substances in diagonally opposite quarters taken in pairs might be compared with those between adjacent quarters taken in pairs. The first cut was made as far as possible to divide equally the area of deeper skin colour so that adjacent quarters in pairs correspond to the half-apple

obtained by cutting once. The results are shown in Table III. Table IV gives the sugar and acid concentration of whole apples, obtained by taking the mean of the four values for the separate quarters, and of half-apples both as the mean concentration in adjacent quarters and in diagonally opposite quarters. These figures are derived from those of Table III. The standard error of the difference between the mean sugar and acid concentrations of samples of nine whole apples, and of the mean differences between the sugar and acid concentration of the two sets of half-apples have been calculated. There is a very much lower error when half-apples are compared instead of wholes, and a reduction in the mean difference between halves from  $0.23 \pm 0.03$  to  $0.07 \pm 0.02$  when diagonally opposite quarters in pairs are compared rather than adjacent quarters in pairs. If diagonally opposite pairs are used fewer apples will therefore be required for each sample for a given degree of significance of the differences between sugar and acid concentrations than if adjacent quarters are used. This advantage is somewhat offset by the additional labour of preparation of the quarter-apples.

TABLE I.

*The Dry Weight of Plugs of Apple Pulp Removed from Different Parts of the Fruit (Bramley's Seedling).*

Apple.	Percentage of fresh weight.					Calyx end.	Stalk end.
	'Blushed' side.	'Unblushed' side.	Intermediate between 'blushed' and 'unblushed'				
1	14.57	12.72	13.88	13.60	14.18	14.55	
2	13.66	12.60	13.21	12.86	14.12	13.25	
3	12.93	11.75	12.58	12.79	13.87	12.69	
4	12.41	11.64	12.66	12.41	13.34	11.68	

TABLE II.

*Dry Weight, Sugar and Acid Contents of Inside and Outside Portions of Apple Pulp (Bramley's Seedling).*

	Percentage of fresh weight.			
	Dry weight.	Reducing sugar.	Sucrose.	Acid.
Apple I				
outside	12.24	6.74	1.73	0.80
inside	11.64	6.59	1.33	0.98
Apple II				
outside	11.98	6.73	1.75	0.76
inside	11.60	6.42	1.31	0.91

*Comparison of sugar and acid loss with the carbon dioxide output.*

This was carried out both by the half-apple method and by using the diagonally opposite quarters in pairs.

TABLE III.

*Total Sugar and Acid Content of each of the Four Quarters of Nine Apples cut through the Calyx and Stalk in Two Planes at Right Angles (Bramley's Seedling).*

Percentage of fresh weight.

Quarters :	1		2		3		4	
	'Blushed' side.				'Unblushed' side.			
	Sugar.	Acid.	Sugar.	Acid.	Sugar.	Acid.	Sugar.	Acid.
Apple A	11·05	1·60	10·90	1·49	10·61	1·51	10·83	1·54
„ B	11·13	1·22	11·52	1·16	10·93	1·07	10·84	1·11
„ C	11·23	1·25	11·75	1·19	11·22	1·19	11·10	1·22
„ D	10·43	1·51	10·10	1·52	10·06	1·47	10·17	1·57
„ E	9·87	1·57	9·55	1·60	9·63	1·54	9·99	1·55
„ F	12·09	1·41	12·19	1·37	11·46	1·36	11·41	1·34
„ G	11·31	1·19	11·54	1·27	11·47	1·34	11·43	1·20
„ J	10·17	1·41	10·66	1·39	10·40	1·40	10·04	1·45
„ K	10·97	1·34	10·84	1·28	10·18	1·30	10·26	1·29

TABLE IV.

*Calculation of Concentration of Sugar and Acid in Different Parts of a Sample of Nine Apples. Data from TABLE III.*

A, Concentration in whole apples, i.e. mean of values for quarters 1, 2, 3, and 4.  
Concentration in half-apples. B<sub>1</sub>, Mean of values for adjacent quarters 1, 4; B<sub>2</sub>, for adjacent quarters 2, 3. C<sub>1</sub>, Mean of values for diagonally opposite quarters 1, 3; C<sub>2</sub>, for diagonally opposite quarters 2, 4.

	A.	Sugar + Acid.		Difference between B <sub>1</sub> & B <sub>2</sub> .	Sugar + Acid		Difference between C <sub>1</sub> & C <sub>2</sub> .
		B <sub>1</sub> .	B <sub>2</sub> .		C <sub>1</sub> .	C <sub>2</sub> .	
Apple A	12.38	12.51	12.25	0.26	12.38	12.42	0.04
" B	12.25	12.15	12.34	0.19	12.17	12.31	0.14
" C	12.54	12.40	12.67	0.27	12.44	12.62	0.18
" D	11.71	11.84	11.58	0.26	11.74	11.69	0.05
" E	11.32	11.49	11.16	0.33	11.30	11.29	0.01
" F	13.16	13.13	13.19	0.06	13.15	13.15	0.00
" G	12.69	12.56	12.81	0.25	12.65	12.71	0.06
" J	11.73	11.54	11.87	0.33	11.74	11.77	0.03
" K	11.86	11.93	11.80	0.13	11.91	11.83	0.08
Mean	12.18	Mean difference		0.23	Mean difference		0.07
Standard Error of		S. E. of mean			S. E. of mean		
Mean	0.193	difference		0.0299	difference		0.0199
S. E. of difference from mean	0.273						

*Material and methods of analysis.* Bramley's Seedling from East Malling Research Station were employed. The apples were from trees on No. 12 stock all of which had received the same cultural treatment. They

were  $2\frac{3}{4}$  to  $3\frac{1}{2}$  in. in diameter and the average weight was 142 gm. The fruit was received 16/10/33 and placed in store at 1° C. until required. The apparatus and methods used were essentially the same as those described by Lall (3). The procedure for cutting and mounting the half-apples in wax was followed exactly. For the quarter-apples the two halves obtained by the first cut were mounted on clock-glasses on which a piece of grease-proof paper was laid before pouring on the hot wax. When the wax was set the half-apple could be lifted off the glass with the wax and paper adhering to the cut surface. On cutting each half into quarters the newly exposed cut surface was placed on melted wax in a clock-glass leaving the first cut surface at right angles to the clock-glass and covered with wax and paper. Each quarter-apple was weighed after being mounted on wax and again at the end of the experiment. The quarters were then carefully detached from the wax and weighed. The weight of the dish and wax was given by difference and used to obtain the weight of each quarter at the beginning of the experiment. A control dish containing wax was placed under the same conditions and no change in weight occurred.

The respiration apparatus was slightly modified by arranging a pump to blow carbon dioxide-free air through the chambers in addition to the suction pump so that air at approximately atmospheric pressure passed through.

The analytical procedure was also slightly modified to eliminate the errors introduced in mixing the cut pulp. For this purpose all the material obtained from each half-apple was extracted with alcohol, none being set apart for dry-weight determinations. Such determinations were carried out by making up the alcoholic extract to a fixed volume and taking an aliquot for drying. The alcohol-insoluble material was dried and weighed and the weight added to that obtained from the alcoholic extract. That similar results are obtained by this method and by drying the pulp directly is shown by the figures of Table V.

The methods of estimation were those described by Archbold (1) except that the Shaffer-Hartman (4) titration for sugar determination was used instead of that of Lane and Eynon.

*Experiment using half-apples.* Twenty apples were removed from the 1° C. store on 3/11/33 and cut and mounted in wax. All the half-apples were then placed in the respiration chamber at 12° C. at which temperature the measurements of carbon dioxide output were to be made. The wounding effect on the respiration rate was found to have ceased by 20/11/33, 17 days after cutting, and one set of halves was then removed and analysed. The corresponding halves were left in the respiration chambers and the carbon dioxide output measured from 20/11/33 to 8/1/34, 7 weeks. This set was then analysed.

TABLE V.

*The Dry Weight of Apple Pulp (Expressed as Percentage of Fresh Weight) Determined by Drying the Cut Pulp at 50° C. (A) and by Drying Aliquot Parts of the Alcoholic Extract at 50° C. (B), and the Residue Insoluble in Alcohol at 100° C. (C).*

	Cut pulp dried at 50° C.	Alcoholic extract dried at 50° C.	Alcohol- insoluble material dried at 100° C.	
	A.	B.	C.	B + C.
Sample 1	15.60	13.80	2.00	15.80
" 2	15.68	13.58	2.01	15.59
" 3	15.60			
Mean	15.63			15.70

TABLE VI.

*Analyses of Corresponding Halves of Bramley's Seedling Apples, Expressed as Percentage of the Fresh Weight on 3/11/33.*

Apples cut and mounted in wax, 3/11/33. Halves A analysed 20/11/33, Halves B 8/1/34. Total carbon dioxide output (gm.) of halves B from 20/11/33 to 8/1/34, at 12° C.

	Total dry wt.	Dry wt. of alcoholic extract.	Total sugar.	Acid (as malic).	Total dry wt.	Dry wt. of alcoholic extract.	Total sugar.	Acid.	CO <sub>2</sub> output.
	Halves A.				Halves B.				
Apple 1	14.72	12.70	9.93	1.39	13.37	11.45	9.31	1.15	1.13
" 3	14.69	12.68	10.25	1.19	13.60	11.76	9.94	0.91	1.00
" 4	15.32	13.26	10.62	1.24	13.77	11.81	9.79	1.00	1.03
" 5	14.99	12.83	10.44	1.21	13.56	11.68	9.85	0.97	0.99
" 6	14.81	12.78	10.43	1.20	13.60	11.76	9.65	0.99	1.22
" 7	14.50	12.58	10.47	1.11	13.42	11.61	9.73	0.92	1.21
" 8	14.48	12.53	10.17	1.21	12.41	10.69	8.95	0.93	1.21
" 9	13.90	11.93	9.84	1.17	12.54	10.75	8.79	0.91	1.17
" 11	14.48	12.56	10.09	1.34	12.51	10.73	8.99	0.99	1.37
" 12	15.84	13.80	11.41	1.10	14.05	12.15	10.39	0.81	1.17
" 13	15.15	13.13	10.69	1.22	13.65	11.71	9.59	0.99	1.16
" 14	14.67	12.60	10.40	1.14	13.44	11.55	9.60	1.00	1.20
" 16	14.67	12.64	10.51	1.25	13.77	11.79	9.65	1.01	1.34
" 17	13.54	11.70	9.53	1.25	12.59	10.80	8.91	1.00	1.24
" 18	16.28	14.04	11.25	1.24	14.53	12.44	10.33	1.00	1.14
" 19	15.17	13.25	10.80	1.19	13.98	12.12	10.02	0.96	1.25
" 20	14.50	12.55	10.12	1.22	12.54	10.83	8.91	0.89	1.41

The results of the analyses and the total carbon dioxide output are shown in Table VI, calculated as percentage of the fresh weight on 3/11/33. Two half-apples became infected with fungi and one sugar solution was lost



by an accident leaving 17 complete results. In Table VII are shown the losses of sugar and acid and of carbon dioxide calculated in terms of carbon from the data of Table VI together with the differences between these losses and the standard error of the mean difference. In fifteen out of the seventeen cases the carbon lost as sugar and acid is the greater, in one case the carbon losses are equal (no. 17) and in one the carbon lost as carbon dioxide is greater (no. 3). The mean difference between these losses is  $0.106 \pm 0.022$ . This difference is nearly five times as great as its standard error and is highly significant.

TABLE VII.

*The Losses of Sugar and Acid and the Carbon Dioxide Output of Bramley's Seedling Apples, given in Terms of Carbon, during 7 Weeks (20/11/33 to 8/1/34) at 12° C.*

The losses are calculated from the data of Table VI.

	Total sugar. 1.	Acid. 2.	Sugar + acid 3.	CO <sub>2</sub> output. 4.	Difference between 3 and 4.
Apple 1	0.248	0.086	0.334	0.307	+ 0.027
" 3	0.124	0.098	0.222	0.274	- 0.052
" 4	0.332	0.083	0.415	0.281	+ 0.134
" 5	0.236	0.085	0.321	0.270	+ 0.051
" 6	0.312	0.074	0.386	0.333	+ 0.053
" 7	0.296	0.067	0.363	0.329	+ 0.034
" 8	0.488	0.102	0.590	0.331	+ 0.259
" 9	0.420	0.090	0.510	0.319	+ 0.191
" 11	0.440	0.124	0.564	0.372	+ 0.192
" 12	0.408	0.102	0.510	0.319	+ 0.191
" 13	0.440	0.083	0.523	0.317	+ 0.206
" 14	0.320	0.050	0.370	0.328	+ 0.042
" 16	0.344	0.087	0.431	0.366	+ 0.065
" 17	0.248	0.090	0.338	0.339	- 0.001
" 18	0.368	0.084	0.452	0.312	+ 0.140
" 19	0.312	0.081	0.393	0.340	+ 0.053
" 20	0.484	0.116	0.600	0.385	+ 0.215
Mean difference					+ 0.106 $\pm$ 0.022

It is evident, therefore, that if a sample of seventeen apples is used, it can be clearly demonstrated by the half-apple method that the loss of sugar and acid in Bramley's Seedling is not accompanied by an equivalent output of carbon dioxide.

*Experiment using pairs of diagonally opposite quarters.* Twenty-five apples were removed from the 1° C. store on 2/2/34 and cut and mounted in wax as already described. The whole set was placed at 12° C. Observation of the respiration rate of five sets of quarters showed that the normal level was reached by 20/3/34, eighteen days after cutting. One pair of diagonally opposite quarters of each of 20 apples was then removed from each chamber and analysed. Measurements of the carbon dioxide output

of the corresponding pairs were made from 20/3/34 to 23/3/34 ( $4\frac{1}{2}$  weeks). At this date the apples were nearing the end of their storage life and unfortunately moulds developed in many cases on removal from the 1° C. store so that complete results were only obtained for nine apples. In this sense, therefore the sample was selected.

During the last week of the respiration measurements of the pairs of quarter-apples the carbon dioxide output of each of five whole apples from the same population was also measured. From the results of Table VIII it may be concluded that the average rate of respiration is unaffected by the cutting into quarters if the period of increased rate immediately after cutting is excluded. The mean rate for five whole apples and for the five sets of quarters is similar.

TABLE VIII.

*Mean Rate of Respiration (mgs./100 gm./hr.) of Whole Apples and of Quarter-apples Five Weeks after Cutting into Quarters.*

The period for whole apples was 316 hours (14/3/34 to 26/3/34) and for quarter-apples 286 hours (10/3/34 to 23/3/34).

Whole apples.	Respiration rate.	Quarter-apples.	Respiration rate.
1	1.203	1	1.380
2	1.095	2	1.365
3	1.302	3	1.139
4	1.107	4	0.989
5	1.353	5	1.380
Mean	1.212		1.250

The results of the experiment with the pairs of diagonally opposite quarters are shown in Tables IX and X, the data correspond to those in VI and VII. It is unfortunate that among these nine results one (no. 21) is very aberrant. The apparent sugar-loss is very small and from the data of Table IV it may be seen that in this population of apples a real value so widely different from the mean loss is extremely unlikely to occur. It is believed that an analytical mistake must account for this result, but as there was no traceable error in the data relating to this apple the figures have been included. The mean difference between the carbon lost as sugar and acid and that lost as carbon dioxide in this set is  $0.044 \pm 0.02$ , this difference is twice its standard error and is significant, but the results show no improvement over the method in which the diagonally opposite quarters in pairs are used instead of half-apples. If the aberrant result is excluded the mean difference becomes  $0.062 \pm 0.010$ . Here the difference is five times as great as its standard error and is highly significant; further the same order of significance is obtained with half the number of apples necessary for the half-apple method.

TABLE IX.

*Analyses of Diagonally Opposite Quarters of Bramley's Seedling Apples, Expressed as the Percentage of the Fresh Weight on 20/2/34.*

Apples cut and mounted in wax 2/2/34. Quarters A analysed 20/2/34, Quarters B on 23/3/34. Total carbon dioxide output (gm.) of halves B from 20/2/34 to 23/3/34 at 12° C.

Total dry wt.			Dry wt. of alcoholic extract.	Total sugar.	Acid (as malic).	Total dry wt.	Dry wt. of alcoholic extract.	Total sugar.	Acid (as malic).	Carbon dioxide output.
2 quarters A.						2 quarters B.				
Apple	3	14.12	12.22	10.23	1.00	12.75	10.96	9.39	0.79	1.318
"	7	14.45	12.43	9.87	1.28	13.72	11.71	9.25	1.07	0.860
"	8	15.36	13.15	10.57	1.25	14.29	12.08	9.90	1.04	1.074
"	9	15.25	13.27	10.84	1.14	14.13	12.19	10.28	0.97	0.767
"	10	13.97	12.13	10.16	1.04	13.04	11.23	9.41	0.83	1.113
"	12	14.28	12.34	9.70	1.29	13.18	11.21	9.03	1.02	1.032
"	13	15.08	13.03	10.71	1.02	13.92	11.93	10.18	0.82	1.029
"	17	13.97	12.05	9.98	1.03	13.02	11.14	9.25	0.85	0.953
"	21	14.70	12.80	10.64	0.99	14.34	12.34	10.47	0.91	0.790
"	24	14.85	12.97	10.90	1.00	13.75	11.90	10.32	0.79	1.020

TABLE X

*The Loss of Sugar and Acid and the Carbon Dioxide Output of Bramley's Seedling Apples, given in Terms of Carbon, during Four-and-a-half Weeks (20/2/34 to 23/3/34).*

The losses are calculated from the data of Table IX.

	Total sugar.	Acid.	Sugar + acid.	CO <sub>2</sub> output.	Difference between 3 and 4.
	1.	2.	3.	4.	
Apple 3	0.336	0.073	0.409	0.359	+0.050
" 7	0.248	0.076	0.324	0.235	+0.089
" 8	0.268	0.077	0.345	0.293	+0.052
" 9	0.224	0.060	0.284	0.209	+0.075
" 10	0.302	0.077	0.379	0.306	+0.073
" 12	0.268	0.098	0.366	0.281	+0.085
" 13	0.212	0.070	0.282	0.280	+0.002
" 17	0.292	0.066	0.358	0.260	+0.098
" 21	0.068	0.032	0.100	0.315	-0.115
" 24	0.232	0.076	0.308	0.278	+0.030
Mean difference					+0.044 ± 0.020
Mean difference (omitting no. 21)					+0.062 ± 0.010

Lall's results for Bramley's Seedling are thus definitely confirmed by these two experiments and it may be regarded as established that from 17 to 30 per cent. of the carbon lost by loss of sugar and acid in these apples does not appear as carbon dioxide.

*Loss of total dry material.*

In the first experiment (half-apples) a mean difference between the loss of dry material and the losses of sugar and acid of  $0.19 \pm 0.05$  was found, and in the second (diagonally opposite quarters) a mean difference of  $0.16 \pm 0.048$ . Both these differences are significant. In these apples, therefore, the loss of dry material is greater than that of sugar and acid, and it is thus unlikely that some of the sugar is converted to a non-volatile product stored in the pulp (see also Lall (3), p. 290). It is not possible to offer any suggestion as to the nature of the products of oxidation other than carbon dioxide without much more data than are at present available.

## SUMMARY.

The concentration of sugar in the apple fruit has been found to be greater on the 'blushed' side than on the 'unblushed' side, and to increase from the stalk end to the calyx end and from inside to outside.

Using the half-apple method described by Lall, the loss of sugar and acid, expressed as carbon, in Bramley's Seedling apples kept at  $12^{\circ}$  C. is shown to be 17 to 30 per cent. *greater* than the carbon lost as carbon dioxide.

The number of apples in a sample necessary to obtain this difference with a given degree of significance can be halved if for each half-apple *diagonally opposite* quarters are taken instead of *adjacent* quarters.

The authors have pleasure in expressing their thanks to Professor V. H. Blackman for his continued interest in the work, and to Mr. R. V. Martin for carrying out the respiration measurements.

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# Morphology of the Stylar Canal in Angiosperms.

BY

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With twenty-seven Figures in the Text.

THE stylar canal<sup>1</sup> or the conducting tissue of the style has received little attention from botanists. It is known that in the case of the hollow styles, as in *Lilium*, *Butomus*, *Agave*, *Erythronium*, *Viola*, *Campanula*, *Sarcodes*, &c., the conducting tissue lines the canal as a glandular layer or in some cases, as in *Anagallis*, fills up a hollow style; but in most cases the style is solid, with the conducting tissue as an axial strand. Coulter and Chamberlain (1) have further suggested that the hollow stylar canal with its lining of conducting tissue represents a primitive angiospermous condition, and the larger development of this tissue has resulted in the prevailing solid style; but they admit the possibility that the reverse may be true, and the hollow stylar canal may be the result of breaking down or rupture from the solid type.

No serious attempt has hitherto been made to trace the relation of the stylar canal with the other parts of the carpel, especially with the vascular system. The purpose of the present paper is to follow this relationship in a few families, the floral anatomy of which the writer has been studying.

It will be useful, however, to indicate first the present position of the anatomy of the carpel. As Eames (2) has shown, the carpel has primarily three traces, a median and two laterals, all arising from separate gaps. The median trace runs along the midrib of the carpel on its dorsal side, and is hence called the dorsal trace or the dorsal bundle. The lateral traces run along the margins of the carpel—along its ventral side—and are hence called the marginal or the ventral traces or bundles. The ovule

<sup>1</sup> The words stylar canal are used here in a rather comprehensive sense. Some authors restrict their use to the hollow type of style. As used here they include both the hollow and the solid types and also the whole pollen-tube conducting tissue of the style and its extensions into the ovary wall which sometimes may reach the very base of the carpel.

traces are derived from the ventral bundles. Sometimes the midrib bundles give rise very near the base of the carpel to two branches, one on either side. These are then called the median laterals. A larger number of traces than three for a carpel has been derived by multiplication from the original three and a smaller number by union or reduction.

The material studied includes the following plants: (1) *Rivina humilis* Linn. (Phytolaccaceae). (2) *Boerhaavia diffusa* Linn., *B. repanda* Willd., *Mirabilis Jalapa* L., and *Bougainvillea spectabilis* Willd. (Nyctaginaceae). (3) *Stellera Chamaejasme* L. (Thymelaeaceae). (4) *Mollugo verticillata* Linn., *Gisekia pharnaceoides* Linn., *Trianthema monogyna* Linn., and *T. pentandra* Linn. (Aizoaceae).

#### PHYTOLACCACEAE.

In *R. humilis*,<sup>1</sup> at the base of the gynaeceum, there are three vascular bundles (Fig. 1). One of these passes into the midrib of the carpel and is the dorsal bundle. The other two ventral bundles unite to form one strand (Fig. 2) which supplies the single basal ovule (Fig. 3). There is no vascular bundle on the ventral side of the ovary, but here the stylar canal makes its appearance (Fig. 3). Both the dorsal bundle and the stylar canal continue up to the apex of the style (Fig. 4).

A glance at Figs. 1-4 leaves no doubt that the stylar canal occupies the place which ordinarily should be occupied by the ventral bundles of the carpel. If these had not been completely exhausted in supplying the ovule, they would have occupied the same place.

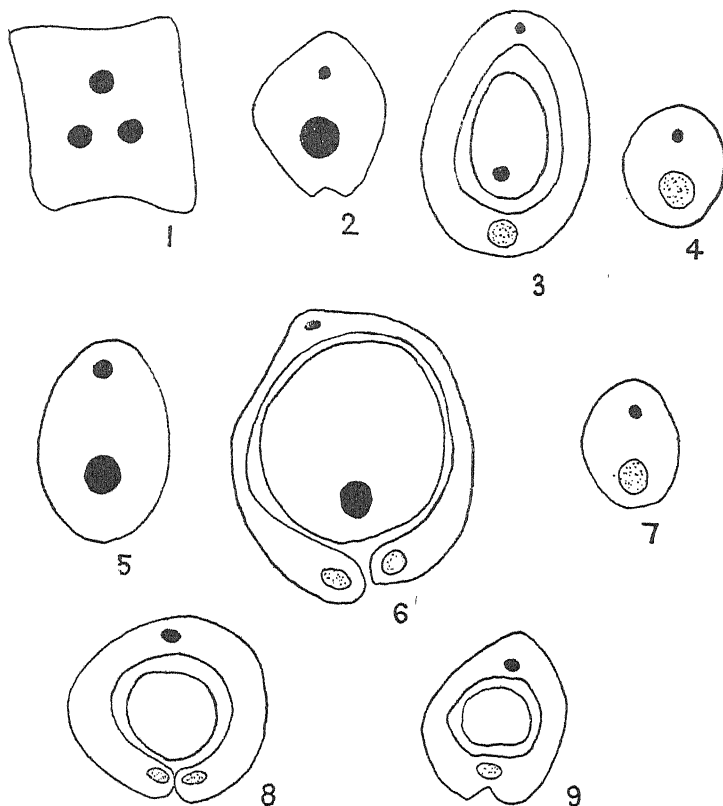
#### NYCTAGINACEAE.<sup>2</sup>

The vascular supply of the carpel and the relation of the stylar canal to the vascular system in the Nyctaginaceae is very similar to that of *Rivina*. Here there are, however, only two bundles at the base of the gynaeceum (Fig. 5), one large and one small. The smaller one is the dorsal bundle and passes on into the midrib of the carpel, the larger one represents the fused ventrals and passes on to supply the ovule. In species of *Boerhaavia* (*B. diffusa* and *B. repanda*) the margins of the carpel near its base never fuse with each other on the ventral side for a short distance, and it remains permanently open at this place. Correlated with this fact, two separate stylar canals make their appearance here (Fig. 6). They occupy exactly the position which is occupied ordinarily by the ventral traces of the carpel. Higher up the two stylar canals join and form one structure. This single stylar canal and the dorsal bundle of the carpel extend right up to the apex of the style, as in *Rivina* (Fig. 7).

<sup>1</sup> Saunders (6) regards the ovary of this plant as bicarpellary. The writer and Rao (4) have shown that it is really monocarpellary as has always been believed. Further details of floral anatomy of this plant are also given in the latter paper.

<sup>2</sup> Complete floral anatomy of some members of this family has been described elsewhere (5).

In *Mirabilis Jalapa* (Fig. 8), although the carpel is not an open structure at the base, there is a distinct line marking the union of its



FIGS. 1-9. *Rivina humilis*; transverse sections of the carpel at different levels from below upwards. 1. Just at the base, showing its three traces. 2. Slightly higher up showing the fusion of the two ventral traces to form one large bundle. 3. Passing of the larger bundle into the ovule and of the smaller into the dorsal wall of the carpel. Styler canal has appeared at the ventral side. 4. Transverse section of the style,—the dorsal bundle and the styler canal continue into this. 5-7. *Boerhaavia diffusa*; there are only two bundles at the base of the carpel (fig. 5). The margins of the carpel are open at the ventral side (fig. 6) and to begin with there are two styler canals. 7. Transverse section of the style. It agrees with *Rivina* in its structure. 8. *Mirabilis Jalapa*, transverse section of the ovary. The carpel here is closed but the line of union of the two margins is quite clear. Styler canals are similar to those of *Boerhaavia*. 9. *Bougainvillea spectabilis*, transverse section of the ovary. Carpel is closed and there is only a single styler canal from the very base. The vascular tissue in all figures is shown in black and the styler canals are dotted.

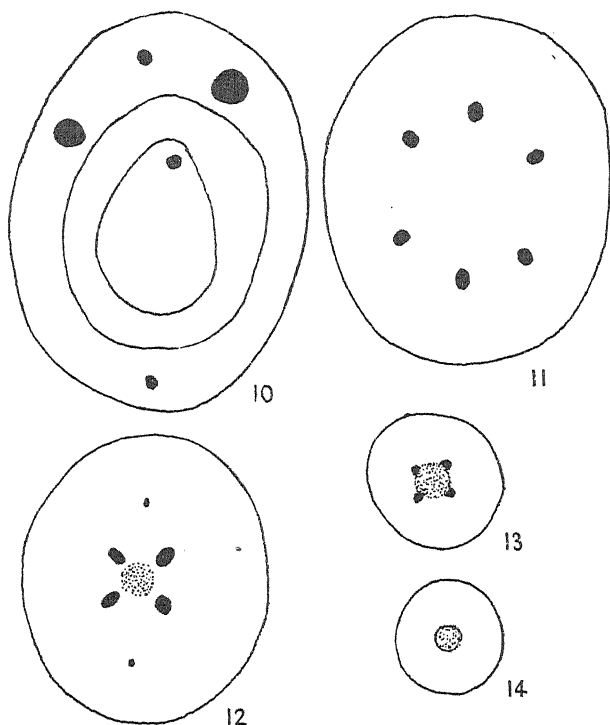
margins, and here also there are two separate styler canals at the base which fuse higher up into one, just as in *Boerhaavia*.

In *B. spectabilis* the carpel is closed at its base, though there is a notch here marking the line of union of the carpel margins. There is a single styler canal from the very beginning, just as in *Rivina* (Fig. 9).

It will be seen that, in every case in the family, the styler canal occupies the place of the ventral traces of the carpel.

## THYMELAEACEAE.

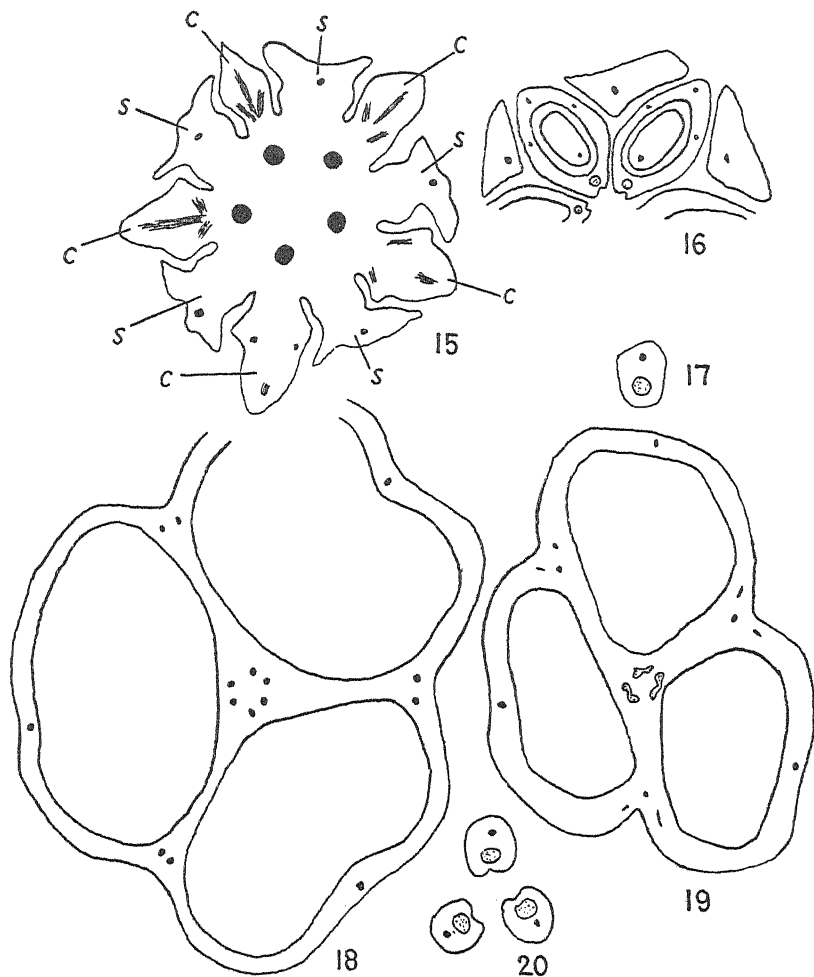
In *S. Chamaejasmae* the gynaecium is usually regarded as monocarpellary, but the writer has shown at another place that it should be really considered bicarpellary (3). Through the greater length of its ovary



FIGS. 10-14. *Stellera Chamaejasmae*; transverse sections of the gynaecium from below upwards. 10. Ovary about its middle, showing four bundles in its wall. 11. Just below the style, showing six bundles. 12. Base of the style; four bundles pass inwards and two remain at the periphery; stylar canal appears in the centre. 13-14. The style; two bundles disappear and the four others merge into the stylar canal. In all figures vascular tissue is shown in black and the stylar canal is dotted.

(Fig. 10) there are four vascular bundles, two of these are larger and supply the ovule and represent the ventral bundles. The other two are smaller and are the midrib bundles. After the vascular supply has been given off to the ovule, each of the larger bundles divides into two so that just below the commencement of the style or at its base there are six bundles, arranged in two groups of three each. In each group there is one dorsal bundle and two ventral or marginal bundles of the carpel (Fig. 11). The dorsal bundles simply disappear at this stage at the periphery of the style, while the four ventral bundles pass inwards (Fig. 12) and merge into the conducting tissue of the style (Figs. 13-14), which is thus seen to be continuous with the ventral traces of the carpels.



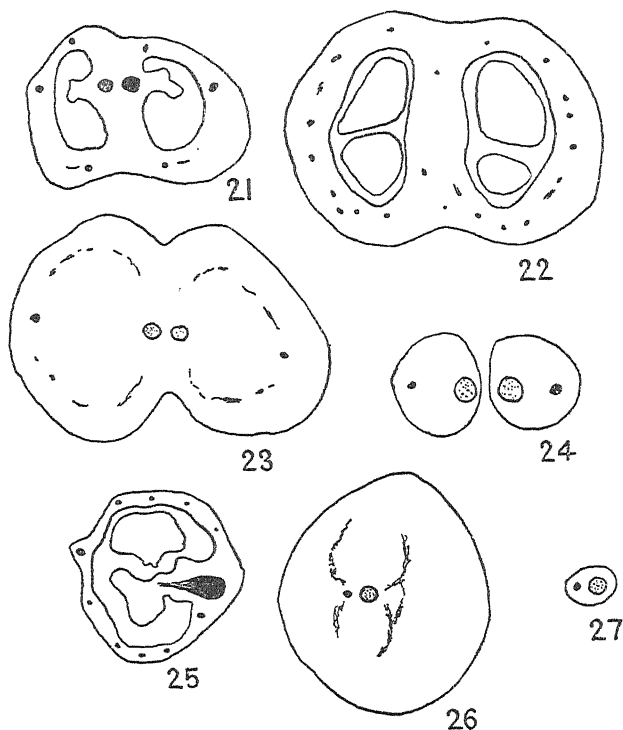


FIGS. 15-20. *Gisekia pharnaceoides*. 15. Transverse section of the thalamus after the perianth leaves have separated, showing the traces of the five carpels marked C. The stamens have not yet separated and are marked S. There are five large bundles in the centre representing the fused ventrals of the carpels. Besides these, in each carpel there is one dorsal bundle and two median laterals. 16. A part of the transverse section of the gynaecium higher up, showing two carpels completely. The ventral bundles have passed into the ovules and the stylar canals have appeared on the ventral side of the carpels. 17. Transverse section of one style, showing the presence of the dorsal bundle and stylar canal. 18-20. *Mollugo verticillata*. 18. Transverse section of the ovary about the middle. There are three carpels, each with one dorsal midrib bundle, two median laterals opposite the septa and two ventrals in the centre. 19. Transverse section on the ovary just below the styles, showing the origin of the stylar canals just at the place of the ventral traces of the carpels. 20. Transverse section of the styles. As before, the vascular tissue in all figures is represented in black and stylar canals are dotted.

#### AIZOACEAE.

In *G. pharnaceoides* the gynaecium consists of a whorl of five free carpels. Just at its base (Fig. 15) before the carpels have separated off, there are five large bundles in the centre, which represent the fused ventral

traces of the carpels, and opposite to these there are the dorsal bundles. Each of these gives rise on its sides to two median laterals. The dorsal bundles pass into the midribs of the carpels, the median laterals on to their



FIGS. 21-7. *Trianthema pentandra*. 21. Transverse section of the ovary. The structure of each carpel is similar to that of *Mollugo* except that the ventrals of each carpel are fused and form only one bundle. 22. The same higher up; the ventral bundles are found to exhaust themselves in supplying the ovules. 23. Shows the origin of the styler canals just in place of the ventral bundles. 24. Transverse section of the two styles. 25-7. *T. monogyna*, transverse sections of the carpel from below upwards. The carpel is many traced, but there is one distinct large ventral bundle and opposite to this a clear dorsal bundle (fig. 25). The styler canal appears at the place of the ventral bundle (fig. 26) and both this and the dorsal bundle continue into the style (fig. 27). As in the previous figures, the vascular tissue is represented in black and styler canals are dotted.

sides, and the fused ventrals into the solitary basal ovules of each carpel. Styler canals appear at this stage on the ventral sides of carpels, so that the condition of each carpel is very similar to that of *Rivina* (Fig. 16), and in the style also, besides the styler canal, the dorsal bundle continues right up to its apex (Fig. 17).

In *Mollugo verticillata* the gynaecium is tricarpeillary syncarpous, with a trilocular ovary, axile placentation, and three distinct styles. A transverse section of the ovary about its middle (Fig. 18) shows six vascular strands, two opposite to each loculus. These are the ventral bundles of the carpels. Opposite to the ventral bundles, in the outer wall of the

ovary lie the midrib bundles and just opposite to the septa, the median laterals. Proceeding higher up, the ventral bundles are found to exhaust themselves in supplying the ovules and slightly higher up in their places appear the stylar canals. There is one for each carpel, and just at the base these are clearly bilobed in a transverse section, and thus show in their form that they are taking the place of the two ventral bundles (Fig. 19). The greater part of the vascular tissue disappears at the top of the ovary, but the midrib bundles continue as very weak strands along with the stylar canals into the three styles (Fig. 20).

In *T. pentandra* the gynaecium is bicarpellary-syncarpous, ovary is bilocular, with central axile placentation and bears at the top two separate styles. About the middle of the ovary (Fig. 21) there are found two large bundles in the axile placenta. These are equivalent to the fused ventrals of each carpel. Opposite to each of these are in the ovary wall, the dorsal or the midrib bundles, and at the sides of the ovary are four median laterals, two belonging to each carpel. The central axile bundles became exhausted higher up in supplying the ovules (Fig. 22). Still slightly higher up two stylar canals make their appearance exactly at the same situation (Fig. 23). Going higher up, the other vascular tissue is found to disappear, but the midrib bundles remain persistent, and one of these and one stylar canal pass into each style (Fig. 24).

*T. monogyna* has a half-inferior monocarpellary pistil with a single parietal placenta bearing many ovules. It receives a large number of traces from the floral axis, but one large one can be at once distinguished at the ventral side, as representing the fused ventrals (Fig. 25). It supplies the ovules. Opposite to it is the midrib bundle. This is also slightly bigger than the other bundles. Higher up the ventral bundle is absorbed in supplying the ovules. All the other traces disappear except the median dorsal. A stylar canal makes its appearance in place of the ventral bundle (Fig. 26), and this and the median dorsal bundle continue into the style (Fig. 27).

#### CONCLUSION AND SUMMARY.

From the description of the various plants given above, it will be seen that in every case the stylar canals are either continuous with the ventral traces of the carpel or make their appearance at, and occupy exactly the situation of, such traces. The conclusion is reached that the stylar canals have been derived from, and represent modified ventral bundles of, the carpels.

The above conclusion appears to be the obvious one. With the origin of the closed carpel with its distinct style and stigma from an open megasporophyll, nothing could be more simple than that the pollen tubes, in order to reach the ovules in the lower part of the carpel, should progress

through some of its vascular bundles. The ventral traces would be the obvious channels since the ovules are borne on the ventral side, and the earliest stylar canals must have originated by a mere cessation of the differentiation of the ventral traces of the carpel at the procambial stage. The solid type of stylar canal is therefore to be regarded as primitive. That this is the form found even at the present day in the majority of angiosperms supports such a conclusion. The hollow type of stylar canal with only a lining of conducting tissue is to be regarded as a derived and more recent condition.

The writer is indebted to Mr. R. A. Laubengayer of Cornell University, Ithaca, New York, for the material of *Mollugo verticillata*.

#### POSTSCRIPT.

The article by Thomas (7) appeared since this paper was in the press.

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# The Ecological and Physiological Action of Ammonium Salts on the Clover Content of Turf.

BY

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With fifteen Figures in the Text.

## INTRODUCTION.

IN two previous papers (2, 3) it has been shown that during the growing season the periodic application of ammonium salts to closely-cut turf favours the growth of the grasses and leads to a reduction of most of the other species. It was demonstrated that this reduction was not due to changes in soil reaction, nor was excess nitrogen responsible, for neither urea nor sodium nitrate was as effective as an ammonium compound. As only ammonium compounds produced a significant diminution of the non-graminaceous plants under conditions not involving a change of soil reaction, it seemed unlikely that such reduction could be ascribed to an increased competition between the grasses and the other species following upon the application of a nitrogenous manure. On the basis of the investigations of Prianshnikow (12, 13, 14, 15), Smirnow (19), Mevius (6), Mothes (8, 9) and Rühland and Wetzel (16, 17, 18), the hypothesis was advanced (3) that the reduction of the species which respond to treatment with ammonium salts was due to a toxic action of the absorbed ammonium ions.

The primary object of the present investigation, which has extended over the period 1930-32, was to obtain further information as to the mechanism of the reduction in the weed content of turf which ammonium compounds produce. Considering a particularly susceptible species, such as *Trifolium repens*, it seemed likely that the injurious action would be related to a depletion of the carbohydrate reserves. The ammonium ions, by combining with the carbohydrates to form 'amide' compounds, would lead, if the uptake of such ions was rapid, to a condition where the depleted reserves would be insufficient for both the replacement of leaf-tissue removed by the constant defoliation (by close-cutting) and for maintaining the concentration of ammonium ions below an injurious level. As a number

of workers have shown that the *Leguminosae*, on the classification of Rühland and Wetzel are typical 'amide plants', it seemed unlikely that organic acids played an important part in the removal of the absorbed ammonium ions. Determinations of the hydrogen-ion concentration of the expressed sap showed that the sap was not markedly acid as with the case of a typical 'acid plant' such as 'rhubarb', but of the same order as other amide species (approximate pH = 6.0). It was argued, on the hypothesis put forward, that if the total available carbohydrate supply could by some means be increased, then the reduction of the clover (*T. repens*) should, to some extent, be lessened; alternatively the same effect would be produced by lowering the rate of absorption of the ammonium ions.

In the experiments large quantities of sucrose alone, and of sucrose with ammonium sulphate, have been added to a sward containing clover. On the hypothesis advanced the addition of sucrose to the ammonium sulphate should lead to a slower rate of reduction of the clover, since the absorption of sucrose with the ammonium ions should reduce the drain on the carbohydrate reserves. The possibility had also to be borne in mind that the uptake of the added nitrogen might be greatly reduced in the presence of sucrose, for such a readily available source of carbon might lead to a large increase in the soil microflora and in consequence to a considerable utilization of the added nitrogen. As the effect of sucrose on the growth of turf, acting either directly, or indirectly through the microflora, was quite unknown some attempt has been made to measure the effect of such treatments by following the changes in botanical composition, and by determining yields by cutting plots at short intervals over periods of two to four months. In addition, the changes in the soil ammonia and nitrate-nitrogen content were followed in 1931. Finally, the initial and final soil reactions were also determined in order to obtain some idea of the acidification produced by the ammonium sulphate dressings in the 1930 experiments, and in 1931 and 1932 to ascertain how successfully this acidification had been counteracted by the addition of calcium carbonate from time to time.

### 1. Changes in Botanical Composition.

All the experiments here described have been carried out on a lawn at this Station which has been cut constantly for the last fifty years; the soil consists of a heavy clay. The chief grasses in the sward were *Agrostis* spp., together with some *Poa trivialis*. Apart from the clover the other 'weeds' were present only in very small amounts.

### EXPERIMENTAL TECHNIQUE.

*Application of fertilizers.* The periodic applications of ammonium sulphate and other substances were made during the spring and summer

months, either at weekly or fortnightly intervals. At each application the ammonium sulphate was added at the rate of 278.5 gm. nitrogen per 1,000 sq. ft., and the sucrose at the rate of 4,540 gm. per 1,000 sq. ft.

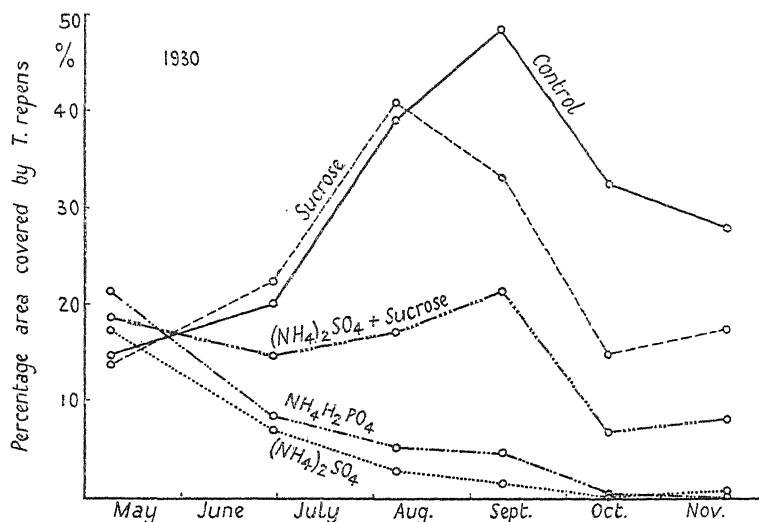


FIG. 1. The effect of various treatments on the percentage area covered by *T. repens*. The rates of application at each weekly dressing were 278.5 gm. nitrogen and 4,840 gm. sucrose per 1,000 sq. ft.

Other nitrogenous fertilizers were applied at rates equivalent in nitrogen to the ammonium sulphate treatment. In order to obtain even distribution the fertilizers were applied in dilute solution by means of a watering can, and subsequently 'watered in' with a hose to prevent damage to the herbage by solutions of high concentration.

*Method of botanical analysis.* The method of botanical analysis has already been described in detail (2, 3). Briefly the method consists in estimating by eye the area covered by each species in a number of small quadrats (6 in.  $\times$  6 in.), ten to thirty quadrats being taken at random on a plot 50–100 sq. ft. in area. Estimates of the error made from time to time showed that duplicate sets of counts agreed on an average within 12 per cent., the largest discrepancies occurring when the content of a species was below 5 per cent. of the total area. The estimations of area covered were carried out periodically during the experiment, and the data collected are capable of statistical treatment by Fisher's (5) methods.

#### EXPERIMENTAL RESULTS.

*1930 experiments.* In 1930 the fertilizers were applied once a week from the beginning of May until the end of October. The treatments consisted of ammonium sulphate, ammonium dihydrogen phosphate, urea,

sodium nitrate, and sucrose, each added separately, together with sucrose added with ammonium sulphate or with sodium nitrate.

The changes in the area covered by the clover are shown in Figs. 1 and 2. Initially the clover contents of the plots were of the same order,

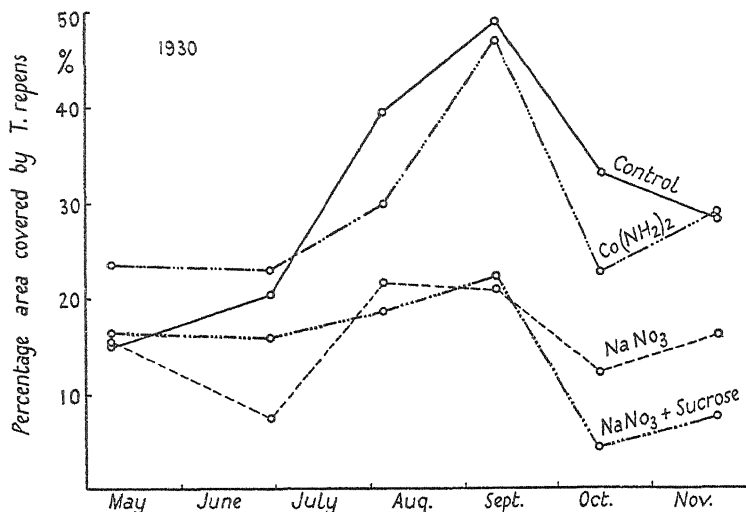


FIG. 2. The effect of various treatments on the percentage area covered by *T. repens*. The rates of application at each weekly dressing were 278.5 gm. nitrogen and 4,840 gm. sucrose per 1,000 sq. ft.

none of the differences being statistically significant. However, the changes brought about by the various treatments were very different, such that at the end of the season the *T. repens* contents relative to the control had been significantly reduced except on the plots receiving urea, or sucrose alone. Moreover, the final content of the plots treated with either ammonium sulphate or ammonium dihydrogen phosphate was significantly less than the content of any of the plots under the other treatments. These results are in agreement with previous findings (3).

All the treatments, with the exception of sucrose, led to a marked increase in the content of *Agrostis* spp., while on the control no change took place. *P. trivialis* was unaffected by the treatments; *Festuca ovina* declined on all plots. Little account need be taken of the changes in the remaining species since they were present only in such small quantities.

**1931 experiments.** In 1931 many of the treatments were the same as in 1930 but urea was omitted, and calcium nitrate replaced sodium nitrate. Additional treatments were included, namely 'nitro-chalk' ( $\text{NH}_4\text{NO}_3 + \text{CaCO}_3$ ), and calcium nitrate at double the usual rate, i.e. 557.0 gm. nitrogen per 1,000 sq. ft. The block of plots was laid down adjacent to those of the 1930 experiment but the fertilizers were applied only once a fortnight from early May until the end of September.



The changes in the clover content of the sward brought about by the various treatments, shown in Figs. 3 and 4, are similar to those produced in 1930. Fitting straight line regression equations showed that neither sucrose alone, nor sucrose with ammonium sulphate, nor calcium nitrate with or without sucrose, had led to significant differences from the control. On the other hand, treatment with ammonium sulphate, 'nitro-chalk', and calcium nitrate at the double rate, brought about a markedly significant reduction in the clover content. Although the regression equations for ammonium sulphate, 'nitro-chalk', and calcium nitrate at the double rate, are not significantly different, the final content of the plot treated with ammonium sulphate is significantly less than those of the other treatments. Since initially the clover content of the plots under these treatments were not significantly different, ammonium sulphate would appear to have caused a greater diminution than the other treatments.

There was an increase in the *Agrostis* spp. on all plots; none of the treatments, however, produced changes significantly different from that of the control. *F. ovina* and the *Poa* spp. tended to decrease in all cases.

*1932 experiments.* These experiments were carried out on an area adjacent to the previous experiments. Some of the treatments were similar to those of the previous year, namely, ammonium sulphate, calcium nitrate, and sucrose alone and with ammonium sulphate. The rates of application were the same as in previous years, i.e. 278.5 gm. nitrogen and 4,540 gm. sucrose per 1,000 sq. ft. Additional ammonium sulphate treatments were also included, the rates of application being 255.3, 232.1, and 185.7 gm. nitrogen per 1,000 sq. ft. The fertilizers were applied fortnightly from early May until mid-September.

The changes in the clover content under the various treatments are illustrated graphically in Figs. 5 and 6. From straight line regression equations fitted to the data it would appear that all treatments, with the exception of sucrose, have brought about a reduction relatively to the control, but the differences between these treatments are not significant. On the other hand, while at the end of the experiment all the plots treated with ammonium sulphate alone had a significantly smaller clover content than initially, treatment with either calcium nitrate or ammonium sulphate together with sucrose had not brought about a significant reduction.

During the experimental period the *Agrostis* spp. increased on all plots, the increases brought about by nitrogenous manuring being significantly greater than the control increase; the *Poa* spp. irrespective of treatment, declined to the same degree.

Comparing the behaviour of the clover under the various treatments, it is seen that the results for the three years are in close agreement. Sucrose alone in each year produced no significant effect. Ammonium sulphate in combination with sucrose was less effective in reducing the

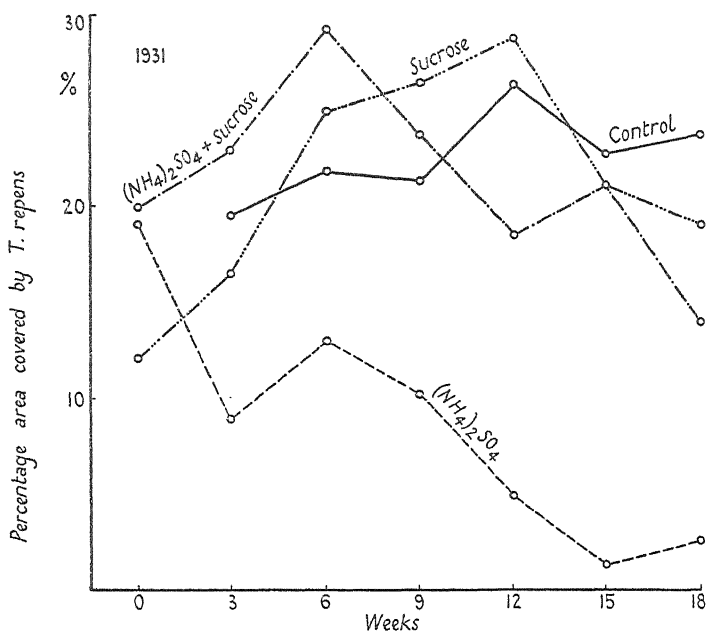


FIG. 3. The effect of various treatments on the percentage area covered by *T. repens*. The rates of application at each fortnightly dressing were 278.5 gm. nitrogen and 4,840 gm. sucrose per 1,000 sq. ft.

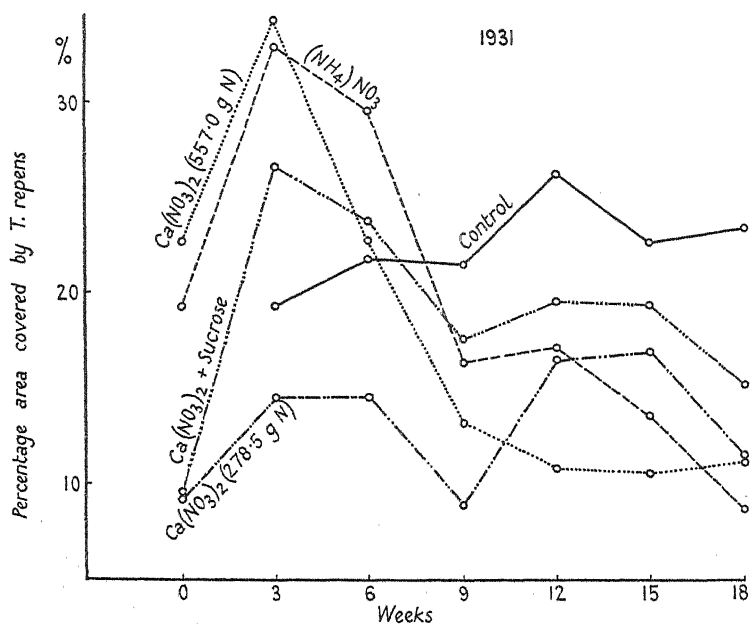


FIG. 4. The effect of various treatments on the percentage area covered by *T. repens*. The figures in square brackets refer to the number of grams of nitrogen applied per 1,000 sq. ft. every fortnight.

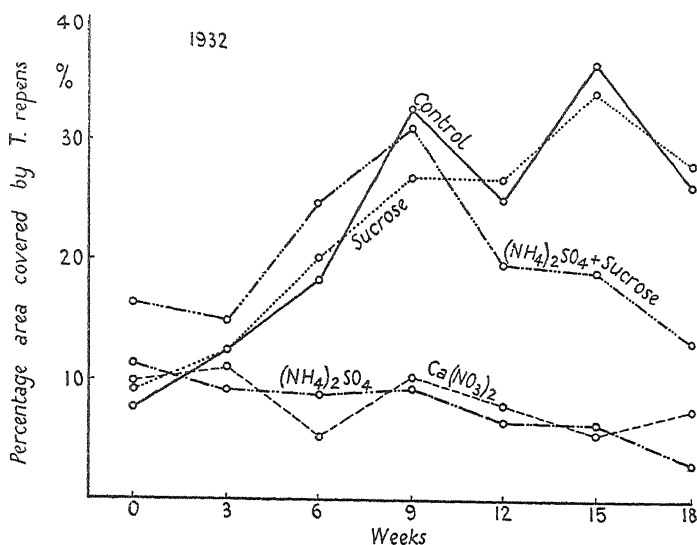


FIG. 5. The effect of various treatments on the percentage area covered by *T. repens*. Both ammonium sulphate and calcium nitrate were applied fortnightly at the rate of 278.5 gm. nitrogen per 1,000 sq. ft. and sucrose at the rate of 4,840 gm.

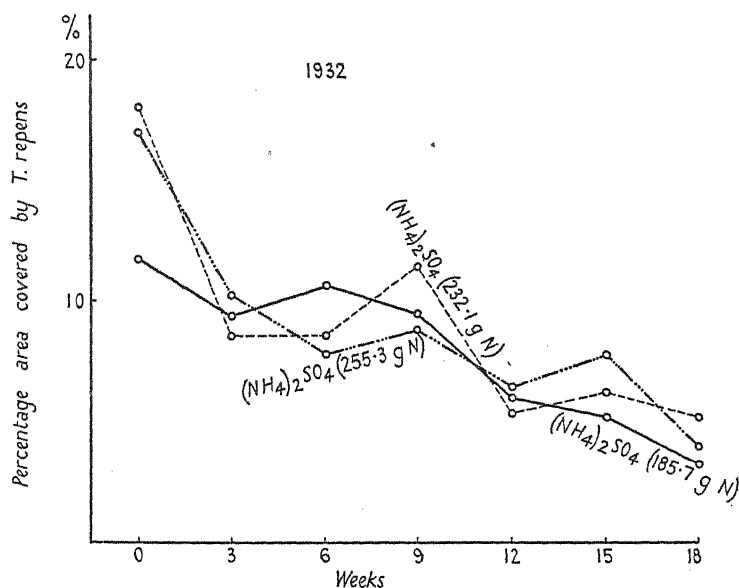


FIG. 6. The effect of treatment with varying amounts of ammonium sulphate on the percentage area covered by *T. repens*. The figures in square brackets refer to the number of grams of nitrogen applied per 1,000 sq. ft. every fortnight.

clover content than ammonium sulphate alone. Sucrose in combination with calcium or sodium nitrate brought about changes similar to those of either calcium nitrate or sodium nitrate without sucrose. In all years, treatment with ammonium sulphate alone led to the greatest reduction. In 1930 the addition of urea to the sward caused no change but sodium nitrate and sucrose and ammonium sulphate and sucrose diminished the clover content. In 1931 and 1932 treatment with calcium nitrate led to no reduction, and in 1931 both calcium nitrate, and ammonium sulphate together with sucrose brought about no diminution. On the other hand, in 1931 'nitro-chalk' and calcium nitrate, both at the double nitrogen rate, reduced the clover, but to a less extent than did ammonium sulphate. Finally in 1932, despite a reduction in the quantity of ammonium sulphate applied, the reduction was not lessened.

## *2. Influence of Treatments on Growth and Uptake of Nitrogen.*

To investigate the influence of sucrose and the various nitrogenous treatments on the growth and uptake of nitrogen by the mixed herbage small plots were cut at weekly or fortnightly intervals over a period of at least ten weeks. At the outset it was realized that it would be difficult to correlate the data with the botanical changes, since the increases in yield over the control brought about by the various nitrogenous substances would be to some extent governed by the varying changes in botanical composition. On the other hand, in order to obtain a pure grass sward on such a long-established turf, pre-treatment with ammonium and iron sulphates would have been necessary. Such treatment would have made the results less comparable with the data of the botanical changes obtained from adjacent areas receiving no pre-treatment. In addition, apart from an initial small clover content (12-17 per cent.), few other weeds were present.

The main object of such experiments was to discover the general effect on the growth of the turf of sucrose alone, and in combination with nitrogenous compounds. If the addition of sucrose to the nitrogenous compound produced changes which were small and of the same order, then there would be strong evidence that sucrose did not act by reducing to a varying degree the available supply of the different nitrogenous fertilizers. If an unequal availability was indicated by the data, it might be claimed that differences in the botanical changes could be ascribed to differences in the degree of competition between the grasses and the clover. Competition would be related to the rate of growth, and this in turn would be to some extent dependent on the available nitrogen in the soil.

In each year the experiments were on similar lines, i.e. a number of replicated plots arranged in random blocks. The plot size varied from 50-100 sq. ft., since plots of a larger size were not practicable in the area available. In 1930 and 1931 the plots were cut every fortnight, and in

1932 once a week. In 1930 the fertilizers were applied weekly, and in 1931 and 1932 fortnightly.

In 1930 the experiment extended over a period of ten weeks, and the four treatments were—control, ammonium sulphate, sucrose, and sucrose added with ammonium sulphate. The rates of application were 278.5 gm. of nitrogen and 4,540 gm. of sucrose per 1,000 sq. ft. The influence of the treatments on the total yield of dry matter for the five cuts is seen in Table I. Sucrose alone had no significant effect on the yield, ammonium sulphate brought about a significant increase of 63 per cent. The addition of sucrose to ammonium sulphate led to a significant reduction of some 15 per cent. when compared to ammonium sulphate alone.

In 1931 four treatments in addition to those of 1930 were included, namely, calcium nitrate and urea, each alone and added with sucrose. The rate of application of nitrogen was somewhat higher than in 1930, being 371.3 gm. per 1,000 sq. ft. As in 1930 (vide Table I), sucrose alone had no significant effect on growth over the experimental period (fourteen weeks) though the reduction was not far short of significance. Sucrose in combination with nitrogen compared with nitrogen treatments alone depressed the yield. The depression for calcium nitrate and sucrose compared with calcium nitrate alone only just failed to be significant, while in the case of ammonium sulphate and urea the significant depressions caused by the addition of sucrose were some 12.5 and 9.0 per cent. respectively. Finally, ammonium sulphate and urea gave a yield just significantly greater than that obtained with calcium nitrate.

TABLE I.

Treatment.	Rate of application of nitrogen.			Yields of dry matter.		
	Gm. per 1,000 sq. ft. per dressing.			Gm. per 1,000 sq. ft.		
	1930	1931	1932	1930	1931	1932
Control . . . . .	—	—	—	8776	25738	22858
Ammonium sulphate . . . . .	278.5	371.3	278.5	14351	44805	36323
"    "    "    "    "    "	—	—	232.1	—	—	33763
"    "    "    "    "    "	—	—	185.7	—	—	34599
Ammonium sulphate and sucrose	278.5	371.3	278.5	12194	39208	33734
Urea . . . . .	—	371.3	—	—	44018	—
Urea and sucrose . . . . .	—	371.3	—	—	38929	—
Calcium nitrate . . . . .	—	371.3	—	—	39201	—
Calcium nitrate and sucrose . .	—	371.3	—	—	34627	—
Sucrose (4,540 gm./1,000 sq. ft.)	—	—	—	8302	22207	23425
Significant difference ( $P = 0.05$ ) .	—	—	—	825	4639	2541

In 1932 the experiment was again modified, ammonium sulphate being added at three nitrogen rates, i.e. 278.5, 232.1, and 185.7 gm. nitrogen per 1,000 sq. ft. These were compared with sucrose alone and with sucrose combined with ammonium sulphate at the rate of 278.5 gm. of nitrogen per

1,000 sq. ft. As in the two previous years, sucrose had no significant effect on the total yield of dry matter from seventeen weekly cuts, while the reduction found with the addition of sucrose to the ammonium sulphate was 7.1 per cent. and not significant. Ammonium sulphate at the rate of 3.0 lb. (278.5 gm. N) per 1,000 sq. ft. gave a significantly greater yield than the 2.5 lb. rate (232.1 gm. N), but not greater than the 2.0 lb. rate (185.7 gm. N). Thus increasing the rate of nitrogen from 2.0 lb. to 3.0 lb. per 1,000 sq. ft. led to no appreciable increase in yield.

Comparing the yields for the three years, 1930-32, ammonium sulphate at the rate of 278.5-371.3 gm. of nitrogen per 1,000 sq. ft. gave increases over the control of 63, 74, and 59 per cent. respectively. Sucrose alone had no significant effect in any year. Sucrose with ammonium sulphate, when compared with ammonium sulphate alone, brought about decreases of 15, 12.5, and 7 per cent. in the three years. In 1931 sucrose added to urea and calcium nitrate produced a similar depression. Finally in 1932 the reduction by a third of the amount of ammonium sulphate added did not lead to any appreciable decrease in yield.

The failure in 1932 to increase the yield in proportion to the increased rate of application of ammonium sulphate, suggested that lack of available nitrogen in the soil was no longer the chief factor controlling growth. That other factors played an important part is suggested by the large variations in yield between consecutive cuts in all three years. The magnitude of these fluctuations is seen in Figs. 7 and 8, where the weekly yields over the 1932 experimental period of seventeen weeks have been plotted. Comparison of the yield figures with the weekly rainfall, hours of sunshine, and mean soil temperature<sup>1</sup> at a depth of 4 in., suggested that hours of sunshine alone showed a marked relationship. Correlation coefficients showed that for rainfall and weekly growth the value of 'r' was small and negative, but that in the case of hours of sunshine and weekly yield, 'r' for the control and for the highest ammonium sulphate rate was +0.1728 and +0.4312 respectively, the partial correlations with the elimination of rainfall being slightly greater, namely +0.217 and +0.4354. The higher correlation coefficient for the nitrogen-treated herbage suggested that sunshine was a more important growth factor under these conditions, and this is confirmed by the fact that the correlation coefficient between increase in growth over the control and hours of sunshine was +0.7052 ( $P > 0.01$ ). This high correlation indicates that the increase in growth due to the addition of nitrogen was largely dependent on sunshine, though it is difficult to say how far this effect is one of temperature or of illumination.

In attempting to correlate hours of sunshine with the weekly yield of the treated plots, it must be borne in mind that a complication arises from

<sup>1</sup> These figures were obtained from the meteorological data of the Meteorological Office sub-station at Jealott's Hill.

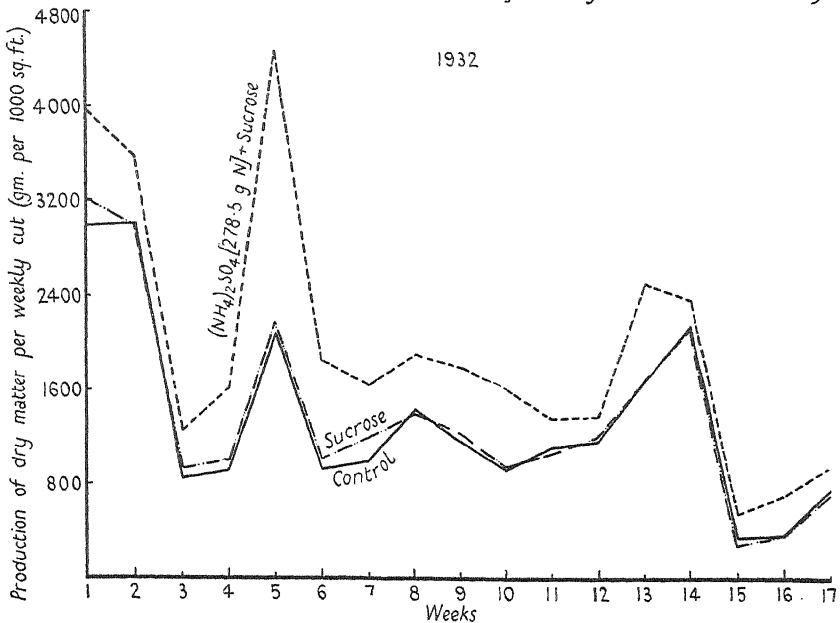


FIG. 7. The influence of sucrose alone and in combination with ammonium sulphate on the production of dry matter from plots cut weekly. The figures in square brackets refer to the number of grams of nitrogen applied per 1,000 sq. ft. The applications were made at the beginning of the 1st, 3rd, 5th, &c., weekly periods.

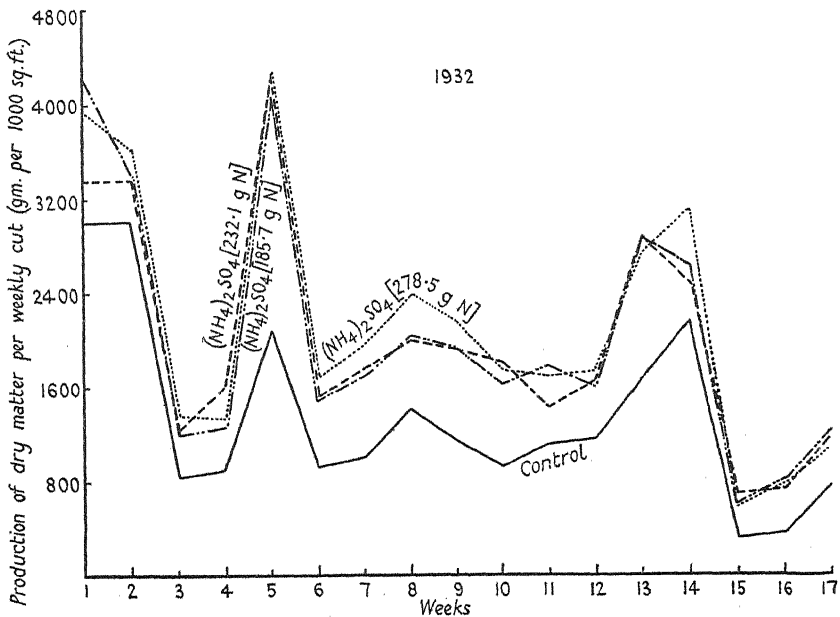


FIG. 8. The influence of ammonium sulphate applied at varying rates on the production of dry matter from plots cut weekly. The figures in square brackets refer to the number of grams of nitrogen or sucrose applied per 1,000 sq. ft. The applications were made at the beginning of the 1st, 3rd, 5th, &c., weekly periods.

the fact that the plots were cut *weekly*, whereas the fertilizers or sucrose were added *fortnightly*. As a result, there was a tendency in the case of the cuts taken a week after the application of ammonium sulphate for the yield to be higher than in the following week when no fertilizer had been applied. This is seen in Table II.

TABLE II.

Treatment.	Rate of application of nitrogen (gm. per 1,000 sq. ft.)	Mean weekly yield (gm. per 1,000 sq. ft.)			
		1st cut, 7 days after fertilizer application.	Per cent. increase over control.	2nd cut, 14 days after fertilizer application.	Per cent. increase over control.
Control	—	1394.0	—	1356.0	—
Ammonium sulphate	278.5	2338.0	67.6	2048.0	51.1
"    "	232.1	2184.0	56.3	1888.0	39.3
"    "	185.7	2298.0	64.5	1838.0	37.0
Ammonium sulphate and sucrose	278.5	2194.0	57.2	1886.0	39.2
Sucrose	—	1464.0	5.1	1374.0	1.3

Table II also shows that the differences between the yields for the first and second weeks after each fertilizer application were most marked for ammonium sulphate plus sucrose, and for the two lighter applications of ammonium sulphate. Thus, the increases in yield for ammonium sulphate at 278.5 and 185.7 gm. nitrogen per 1,000 sq. ft. were in the first weeks 67.6 and 64.5 per cent, respectively, but in the second weeks 51.1 and 37.0 per cent. These figures suggest that the amount of nitrogen stored in the roots is not sufficient to maintain growth at a maximum during the second weeks. They also indicate that the nitrogen added must disappear rapidly from the soil or become unavailable, so that the effective concentration left by the second week is so low that the rate of absorption controls growth.

In addition to the differences in yield in the first and second weeks the nitrogen contents of the herbage from the plots treated with ammonium sulphate also showed similar fluctuations. It is seen from Figs. 9 and 10 that the nitrogen contents of the herbage from the three ammonium sulphate treatments showed very definite maxima in those cuts taken a week after the fertilizer was applied, but in the case of ammonium sulphate and sucrose the relationship was not so distinct. The increases in nitrogen content brought about by nitrogenous manuring were initially quite large, in spite of the already high nitrogen content of the herbage on the control. With time, however, the differences became less marked owing to the gradual fall in nitrogen content of the herbage from the treated plots and the gradual rise in nitrogen content of the control. This rise on the control, it is suggested, was due in some measure to the marked development of the clover (vide Fig. 5), which contains a higher nitrogen content than the grasses (Askew (1)).



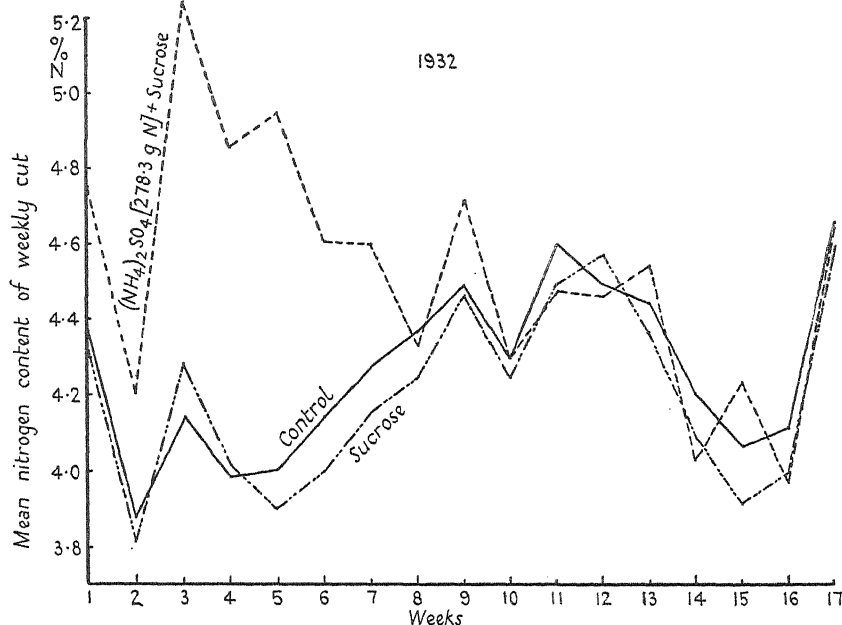


FIG. 9. The influence of sucrose alone and in combination with ammonium sulphate on the nitrogen content of the produce from plots cut weekly. The figures in square brackets refer to the number of grams of nitrogen or sucrose applied per 1,000 sq. ft. The applications were made at the beginning of the 1st, 3rd, 5th, &c., weekly periods.

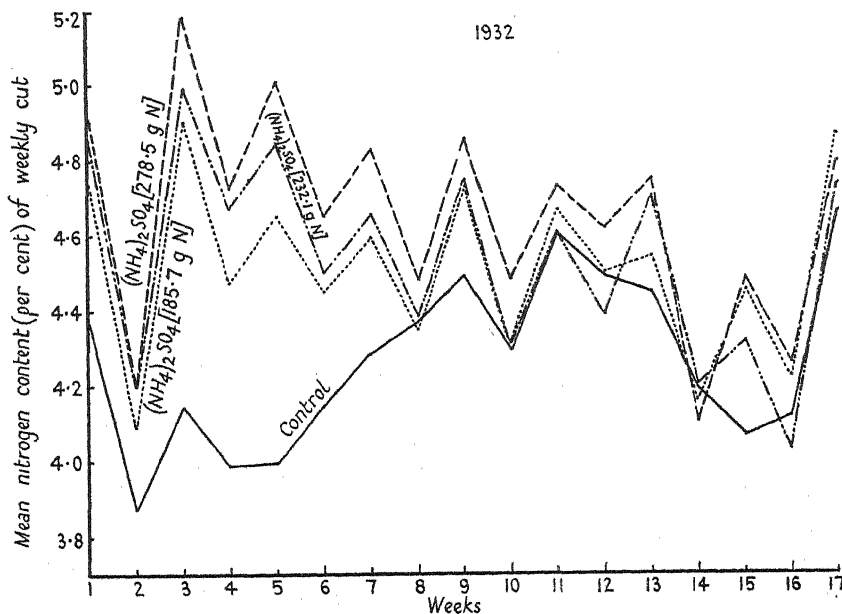


FIG. 10. The influence of ammonium sulphate applied at varying rates on the nitrogen content of the herbage from plots cut weekly. The figures in square brackets refer to the number of grams of nitrogen applied per 1,000 sq. ft. The applications were made at the beginning of the 1st, 3rd, 5th, &c., weekly periods.

*Uptake of nitrogen.* From a knowledge of the yields of dry matter and of nitrogen content of the herbage it is possible to determine how much of the added nitrogen can be accounted for by uptake by the roots and translocation to the aerial organs. In order that data susceptible to statistical analysis might be available on this point the nitrogen content of the herbage from each plot for each cut was determined separately, involving in 1931-2 some six hundred Kjeldahl determinations. The figures for the total amount of nitrogen removed in the herbage, the mean nitrogen content of the herbage, and the percentage of the added nitrogen so recovered are seen in Table III.

TABLE III.

Treatments.	Rate of <sup>1</sup> application of nitrogen (gm. per 1,000 sq. ft.)		Total amount of nitrogen removed in herbage (gm. per 1,000 sq. ft.)		Percentage recovery of added nitrogen.		Mean nitrogen content of dry matter per cent.	
	1931	1932	1931	1932	1931	1932	1931	1932
Control	—	—	1096.6	973.2	—	—	4.27	4.26
Ammonium sulphate	371.3	278.5	2238.9	1690.4	43.0	28.9	5.01	4.65
"    "	—	232.1	—	1544.4	—	27.3	—	4.54
"    "	—	185.7	—	1561.0	—	35.1	—	4.51
Ammonium sulphate and sucrose	371.3	278.5	1899.4	1545.2	30.3	22.7	4.85	4.53
Urea	371.3	—	2224.4	—	42.8	—	5.06	—
Urea and sucrose	371.3	—	1869.4	—	29.6	—	4.81	—
Calcium nitrate	371.3	—	1875.3	—	30.3	—	4.86	—
Calcium nitrate and sucrose	371.3	—	1606.6	—	19.3	—	4.69	—
Sucrose	—	—	905.5	983.8	—	—	4.10	4.20
Significant difference ( $P = 0.05$ )	—	—	222.0	123.0	—	—	—	—

In 1931 the amount of nitrogen removed in the herbage treated with urea or ammonium sulphate was more than double that of the control, and significantly greater than that removed from the plots dressed with calcium nitrate. Sucrose alone had no significant depressing effect on the nitrogen-uptake but added to the nitrogenous compounds it brought about significant reductions, the percentage decrease varying from 15 to 16 per cent. In 1932 sucrose again had no effect on the nitrogen uptake, but when added to ammonium sulphate it brought about a reduction of 9 per cent. The application of ammonium sulphate at the highest rate led to a significantly greater nitrogen uptake than at the lower rates.

The percentage recovery figures show that only a small proportion of the nitrogen given during the experiment is found in the cut herbage; the greatest recovery in 1930 was 43 per cent., and in 1931, 35.1 per cent.

<sup>1</sup> There were seven applications in 1931 and eight in 1932.

These figures do not, however, take into account the amounts of the added nitrogen present in the roots of the treated turf at the time of the final cut.

As it has been shown by Evans (4) that ammonium sulphate depresses rather than increases root growth it is unlikely that any of the added nitrogen present in the roots would have been utilized for new root formation. It should therefore be stored in some readily available form, though for the reasons already given the amount can hardly be large. Further evidence against any considerable accumulation is the fact observed in 1932 that towards the end of the experimental period the differences between the nitrogen contents of the herbage cut a week and a fortnight after the fertilizer applications were more pronounced than earlier in the experiment.

Neither at the end of the experimental period in 1931 nor in 1932 was any attempt made to determine the actual amount of nitrogen in the root. In previous attempts it has been found impossible to separate from the dense mat in the first two inches of soil the living from the dead roots. In addition, in order to obtain statistically accurate information a separate estimate of the roots in each plot would have been necessary, and this would have involved taking several hundred cores.

Estimations in 1933 and earlier have shown that the amount of dead and living roots in the first three inches of soil varies between 40 and 65 kilograms of dry matter per 1,000 sq. ft. These estimates were confined to the first three inches of soil, since by far the greater part of the roots are restricted to this layer under a system of constant cutting. The estimates are in agreement with the data of Sprague (20), who found that in closely cut swards of *Agrostis* spp. the maximum development varied between 57 and 27 kg. per 1,000 sq. ft., while 88–98 per cent. of the roots were found in the first three inches. He showed, in addition, that while ammonium sulphate alone increased root development ammonium sulphate plus 'light liming' brought about a depression. In the present investigation there is little evidence that ammonium sulphate, with or without lime, had any effect on root growth.

In 1933 a series of plots was dressed with twelve *weekly* applications of ammonium sulphate, each at a rate of 464 gm. nitrogen per 1,000 sq. ft. At the end of this period, analysis of the dried root-material from the treated and control plots showed only 0.2 per cent. difference in nitrogen content. This small difference, in spite of the rate of application being more than double that used in 1931, was probably due to the large proportion of dead roots in the samples.

From these data it would appear that at the end of the 1932 experiment the added nitrogen stored in the treated roots could not, at an outside estimate, be more than 100–200 gm. per 1,000 sq. ft., that is to say, only some 7–14 per cent. of that added as ammonium sulphate. On such a

basis the total recovery of the added nitrogen by the sward in 1932 did not exceed 50 per cent. with any treatment. In 1931, when the cuts were made fortnightly, it is possible that as much as 60 per cent. of the nitrogen was removed by the plants.

### 3. *The Changes in the Ammonia and Nitrate-nitrogen Content of the Soil.*

In order to determine to what extent sucrose alone or in combination with the nitrogenous fertilizers depressed the ammonia- and nitrate-nitrogen content of the soil, determinations of these were carried out over a period of ten weeks in 1931. These estimations were made on the plots of the 1931 experiment previously described, in which the plots were cut fortnightly for yield determinations. The manurial treatments, seven in all, consisted of ammonium sulphate, urea, calcium nitrate, and sucrose, together with sucrose added to each nitrogenous compound.

*Experimental technique.* The plots were sampled weekly, four and eleven days after each fortnightly application of the fertilizers, and eight and one day prior to each fortnightly cut. (In the case of the first of the five applications, the sampling was carried out a week after the initial application.) Each fertilizer dressing corresponded to 48.4 parts of nitrogen per million parts of dry soil in the top three inches. The method of soil sampling consisted in taking at random on each plot five small cores (2 in. diameter) the length of each core being 3 in. A greater depth of sampling was considered unnecessary, since it was found, as already stated, that the percentage of the root below three inches is very small. In the laboratory the cores were passed through a 1 mm. sieve to remove the larger roots and small stones, and the ammonia and nitrate nitrogen determined by Olson's (10) methods. The time and labour involved did not allow of estimations being carried out for each plot separately, so the cores from the four replicates of each treatment were bulked. However, some indication of the sampling error is forthcoming from the determinations made on the plots destined for the eight treatments prior to the application of any fertilizers. For both ammonia and nitrate the variance was very small, the mean contents being  $23.3 \pm 0.52$  and  $3.16 \pm 0.22$  parts per million of ammonia and nitrate nitrogen respectively, or, expressing the standard errors as percentages, 2.24 and 6.7. Further indications that the method of sampling was satisfactory are seen in Fig. 11, where the fluctuations in ammonia nitrogen on the plots receiving sucrose are seen to follow the control changes very closely.

*Changes in ammonia and nitrate nitrogen.* The data for the weekly determinations of ammonia and nitrate nitrogen are shown graphically in Figs. 11, 12, and 13. In addition the rainfall for each seven-day period has been included.

In Fig. 11 it is seen that sucrose alone has not brought about a marked depression, relative to the control, in either the ammonia or nitrate content of the soil. Initially the addition of either ammonium sulphate alone or together with sucrose had little effect. After the fourth week, i.e. following

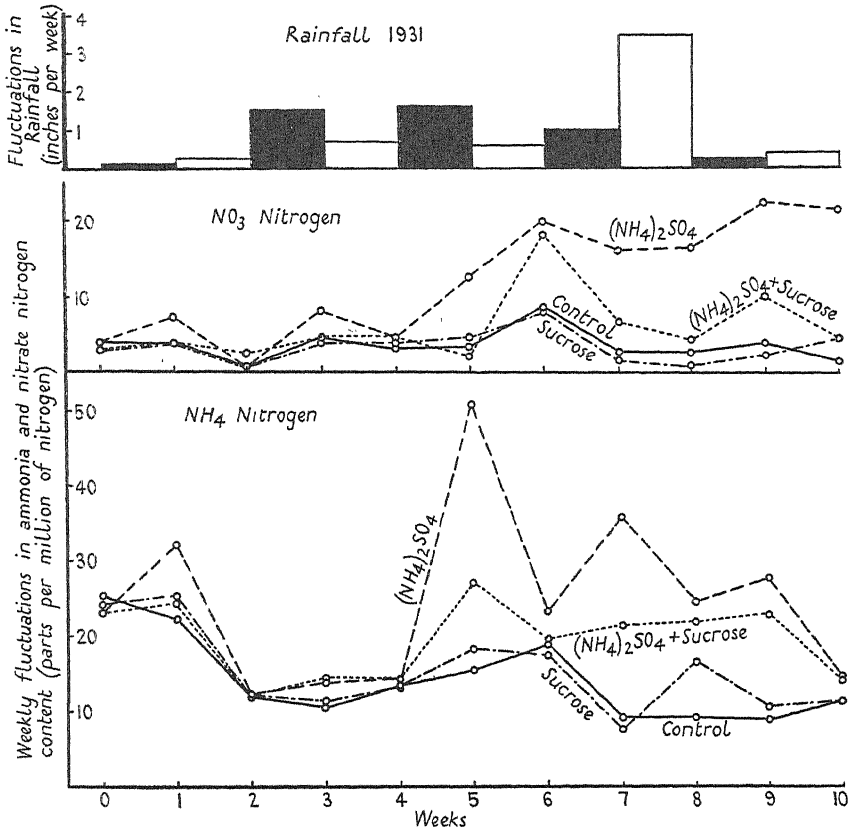


FIG. 11. The effect of various treatments on the ammonia and nitrate-nitrogen concentrations in the soil. The applications were made at fortnightly intervals four days prior to the determinations made at the end of the 1st, 3rd, 5th, &c., week. Each nitrogenous dressing was equal to the addition of 48.4 parts per million of nitrogen in the top 3 in. of air dry soil. The rainfall over the weekly periods is also shown.

upon the third fertilizer application, there was an increase in the contents of both ammonia and nitrate nitrogen. The addition of sucrose, combined with ammonium sulphate, led to a smaller increase over the control in both forms of nitrogen. Subsequent to the third and later applications both ammonium sulphate treatments show marked peaks in the ammonia concentration in the samples taken four days after the application.

The changes with the urea treatments do not show such definite differences from the control as with the ammonium sulphate treatments. Urea alone (vide Fig. 12) brought about only small increases in the ammonia and nitrate concentrations, which did not become appreciably

greater with successive fertilizer applications. The increases in ammonia and nitrate as a result of the addition of urea and sucrose together, cannot be considered significantly different from the control.

The application of calcium nitrate alone raised the soil nitrate concen-

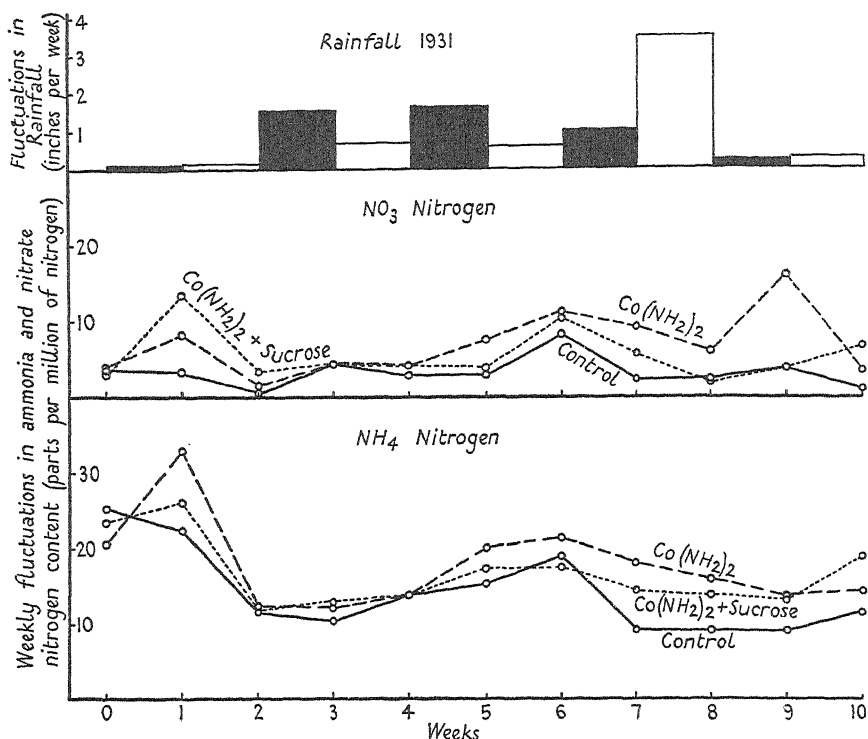


FIG. 12. The effect of various treatments on the ammonia- and nitrate-nitrogen concentrations in the soil. The applications were made at fortnightly intervals four days prior to the determinations made at the end of the 1st, 3rd, 5th, &c, week. Each nitrogenous dressing was equal to the addition of 48.4 parts per million of nitrogen in the top 3 in. of air dry soil. The rainfall over the weekly periods is also shown.

tration by a surprisingly small amount (Fig. 13). Until the eighth week the differences from the control were never more than 12 p.p.m., but in the ninth week there was a sudden increase followed by an equally rapid fall in the next week. The addition of sucrose again depressed the nitrate level; the concentrations, except in the fifth and sixth weeks, were the same as the control. Manuring with either calcium nitrate alone or combined with sucrose had little effect on the ammonia nitrogen content.

The very heavy rainfall, particularly in the seventh week (3.41 in.), probably caused a considerable amount of nitrate nitrogen to be leached from the surface layers. On the other hand, the high rate both of evaporation and transpiration during July and August would again draw up some of this nitrogen into the top three inches. It might be suggested that the

low nitrate level on the calcium nitrate treated plots was entirely due to leaching, but this suggestion is not in agreement with the fluctuations in

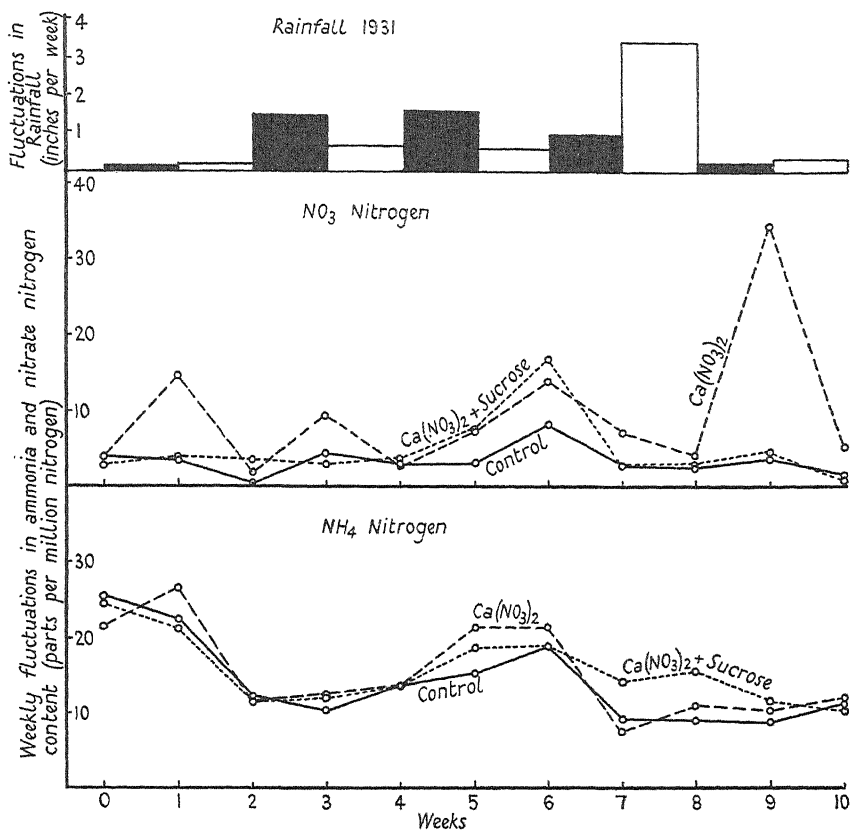


FIG. 13. The effect of various treatments on the ammonia- and nitrate-nitrogen concentrations in the soil. The applications were made at fortnightly intervals four days prior to the determinations made at the end of the 1st, 3rd, 5th, &c., week. Each nitrogenous dressing was equal to the addition of 48.4 parts per million of nitrogen in the top 3 in. of air dry soil. The rainfall over the weekly periods is also shown.

the nitrate content of the ammonium sulphate treated areas, which show little association with rainfall.

It has been shown that less nitrogen was removed in the cut herbage from the plots receiving calcium nitrate than from those receiving ammonium sulphate (vide Table III). It might therefore be expected that there would be a higher concentration of inorganic nitrogen in the soil treated with calcium nitrate, but this is not the case. A comparison of the ammonia and nitrate fluctuations with the uptake of nitrogen in the first of the five cuts (vide Table IV) suggests that these nitrate losses cannot all be ascribed either to leaching or uptake by the plant. At the time of the first cut twice as much nitrogen was removed in the herbage treated with ammonium

sulphate as in that treated with calcium nitrate. Yet in a period when the rainfall was negligible the increases in the ammonia and nitrate contents of the soil, brought about by the two treatments, in spite of this difference in uptake by the plants, were of the same order, namely 13.3 p.p.m. of nitrogen for ammonium sulphate and 15.3 p.p.m. for calcium nitrate.

TABLE IV.

*Recovery of Added Nitrogen in Fortnightly Cuts.*

Treatment.	(gm. per 1,000 sq. ft.)				
	1st.	2nd.	3rd.	4th.	5th cut.
$(\text{NH}_4)_2\text{SO}_4$ . . . . .	88.1	118.1	229.7	179.7	174.5
$(\text{NH}_4)_2\text{SO}_4$ + Sucrose . .	42.8	93.6	153.3	144.4	115.8
$\text{Ca}(\text{NO}_3)_2$ . . . . .	39.7	128.9	131.1	134.7	137.7
$\text{Ca}(\text{NO}_3)_2$ + Sucrose . .	55.0	60.0	111.2	86.9	33.6

A week prior to each cut nitrogen was added at the rate of 371.3 gm. per 1,000 sq. ft.

In Fig. 11 it is seen that not until after the third application of ammonium sulphate did the ammonia and nitrate concentrations rise appreciably above the control. On the other hand, the nitrogen removed in the third cut (vide Table IV) was equal to the nitrogen removed in both the first and second cuts, and it would therefore be expected that in the third cut the ammonia and nitrate concentrations would be lower, not higher, than in the previous weeks. This anomalous result can hardly be attributed to leaching losses, since the rainfall in the week following the third application was just as great as in the week following the second application.

In relating the nitrogen removed in the cuts to the soil inorganic nitrogen, the assumption has been made that the nitrogen removed in the herbage is proportional to the total uptake of nitrogen by the plant. No objection to this assumption can be raised when comparing the amounts of nitrogen removed in the initial cut, since otherwise it is necessary to assume that there are marked differences in the partition of the nitrogen between the root and aerial tissue with the two nitrogenous fertilizers. In the case of the ammonium sulphate treatment, where the nitrogen in the third cut is related to the nitrogen in the first and second cuts, it is possible that there might initially be an accumulation of nitrogen in the roots resulting in a period of delay before it appeared in the shoot. The evidence of the 1932 experiment, however, indicates that the storage of nitrogen in the roots is small and there is little evidence of any lag period.

#### 4. *Changes in Soil Reaction.*

It has been demonstrated previously (2, 3) that repeated application of ammonium sulphate may bring about considerable increases in the



acidity of the soil and that these increases vary with soil depth. In all the present studies, samples were taken for determination of the hydrogen-ion concentration at the beginning and end of each experiment. It was found in 1930 that under the experimental conditions the ammonium

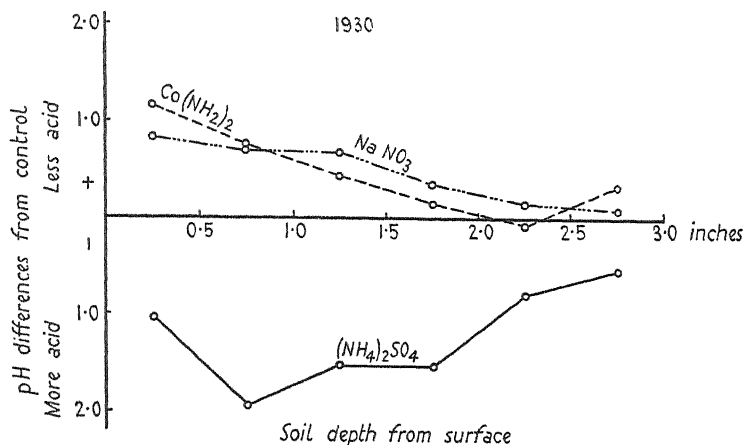


FIG. 14. The changes in soil reaction produced at different depths by the treatments. The results have been plotted relative to the seasonal fluctuations on the control.

sulphate led to very marked changes in soil reaction. In 1931 and 1932, in order to counteract this acidification some days after each ammonium sulphate application, calcium carbonate was added at the rate of 85 gm. for every 100 gm. of ammonium sulphate.

*Experimental technique.* The method of soil sampling has been described in an earlier paper (2). Cores to a depth of 3 in. are obtained by driving in small cylindrical tins. The cores can be easily cut into six transverse half-inch sections. The pH determinations, some six hundred in all, were carried out by the quinhydrone method, using gold electrodes.

*1930 experiment.* In Fig. 14 the differences in pH values between the treated soils and the control have been plotted for the ammonium sulphate, urea, and sodium nitrate treatments. The results have been expressed in this way since there was a decrease in pH on the control between the beginning and end of the experiment. The data show that there was a large increase in acidity where ammonium sulphate was added, and that the greatest acidification took place in the second half-inch. Sodium nitrate, on the other hand, tended to increase the pH of the originally slightly acid soil. Urea also produced less acid soil conditions.

*1931-2 experiments.* The effect of adding calcium carbonate with the ammonium sulphate is shown in Fig. 15, where the data have been plotted in the same way as in Fig. 14. In 1931, the addition of calcium carbonate prevented the hydrogen-ion concentration increasing appreciably, but in 1932 some acidification still took place. In both 1931 and 1932, calcium

nitrate appeared to have a slight acidifying action in the soil. Urea also led to a fall in pH, this result being in conflict with the findings of 1930 and previous evidence (3), but in agreement with the investigations of Pierre (11).

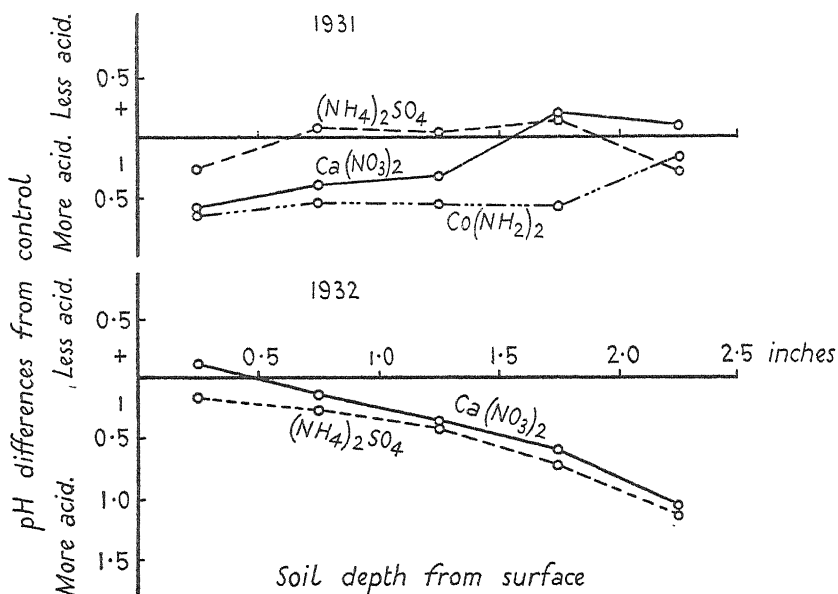


FIG. 15. The changes in soil reaction produced at different depths by the treatments. The results have been plotted relative to the seasonal fluctuations on the control.

### DISCUSSION.

The evidence drawn from the botanical changes which result from the application of nitrogenous compounds with or without the addition of sucrose support the view that the greater reduction of clover (*T. repens*) brought about by ammonium compounds as compared with nitrate salts is due to a toxic action of the ammonium ions. In this view the ions on absorption either combine with the carbohydrates to such an extent as to deplete the reserves, or with lowered reserves the ions reach a concentration injurious to the plant; it is likely that both effects occur. On such a hypothesis the addition of sucrose with the ammonium sulphate should reduce both these effects and so diminish the reduction of the clover; this is the result observed. Sucrose alone had no effect, and sucrose added to either calcium or sodium nitrate had little effect on their action; such results would also be expected on the hypothesis.

In view of the fact that the Leguminosae do not in general respond to nitrogenous manuring, cf. Williams (21),<sup>1</sup> it might be argued—on the

<sup>1</sup> The response of *T. repens* to nitrogenous compounds is being investigated in pot culture. Preliminary results on the whole confirm the work of Williams.

basis of the data from plots cut frequently in 1931—that the greater reduction of clover produced by ammonium sulphate was due to a greater stimulation of the grasses by ammonium sulphate than by calcium nitrate. On the same assumption it might be put forward that the addition of sucrose would reduce, through the action of the microflora, the availability to the grasses of the added nitrogen, and thus lessen the intensity of competition between the grasses and the clover. There are, however, a number of findings which are not in agreement with this suggestion. In the first place the addition of sucrose to each nitrogenous compound brought about a small reduction in yield which was of the same order in each case. Therefore in 1930 and 1932 when sodium and calcium nitrate diminished the clover content to some extent it would be expected that the addition of sucrose would lead, as in the case with ammonium sulphate, to a smaller reduction in the clover content; this, however, was not the result in either year. A further objection to the ‘crowding out’ hypothesis is the result of 1931 when calcium nitrate applied at the double rate (457 gm. nitrogen per 1,000 sq. ft.) was less effective in reducing the clover than ammonium sulphate at the single rate. Finally, in 1932, it has been shown that ammonium sulphate applied at the rates of 187.5 and 232.1 gm. of nitrogen per 1,000 sq. ft. led to a greater reduction in the clover content than sucrose and ammonium sulphate (278.5 gm. nitrogen per 1,000 sq. ft.) despite the increases in yield over the control being of the same order.

In the face of these objections it would seem untenable to maintain that sucrose added to ammonium sulphate lowers the reduction of clover by decreasing the competitive power of the grasses. As has been pointed out previously, it must be borne in mind in any interpretation of the yield-data that the experiments were not conducted on a pure grass sward but on turf containing small quantities of clover, with the result that with different treatments there were differences in the botanical changes. The presence of clover in such small proportions does not, however, appear to affect the conclusions, for in spite of seasonal differences in the botanical changes the yield results show a very marked agreement for the three years. In addition, the closeness in the correlation between the weekly fluctuations in yield, irrespective of treatment, suggests that factors other than differences in the botanical changes were largely controlling growth.

The experiments have shown that by adding sucrose with the nitrogenous fertilizers not only has the yield been depressed in each case, but also the nitrogen content of the herbage has been reduced, indicating that less nitrogen was absorbed from the soil. The periodic determinations of ammonia- and nitrate-nitrogen content of the soil indicate that these reductions have been brought about by sucrose decreasing both the concentrations of ammonia and of nitrate-nitrogen. In the case of the ammonium sulphate treatment, the ratio of the ammonia to nitrate nitrogen in the soil

has been altered, so that the nitrate level has been decreased more than the ammonia level, while with calcium nitrate the nitrate level alone has been diminished. It would therefore be expected that by depressing the nitrate level to a greater extent, the addition of sucrose with ammonium sulphate would lead to a greater proportion of ammoniacal nitrogen being absorbed by the roots.

As clover does not respond to nitrogenous manuring any nitrogen compounds absorbed by the roots should accumulate in the tissues and not be utilized for fresh tissue formation, as in the case of the grasses. Thus any marked absorption of ammonium ions by clover must lead to a greater call on the reserve sugars than in the case of the grasses. The experiments have shown that the increases in nitrogen content of the cut herbage as the result of manuring have been large. Though the increases in nitrogen content of the clover and grasses were not determined separately, it would seem probable, on *a priori* grounds, that although the amounts absorbed might be different, the uptake of nitrogen by the grasses and clover would be influenced in the same direction by the same conditions. Askew (1), comparing the chemical composition of *Lolium perenne* and *T. repens* in a sward never allowed to exceed 2-3 in. in height, found that samples analysed three weeks after the application of ammonium sulphate (239.3 gm. nitrogen per 1,000 sq. ft.) showed on an average an increase in nitrogen content of 7.8 per cent. in the case of *T. repens* and 22.3 per cent. in the case of *L. perenne*. An experiment at Jealott's Hill carried out in the winter of 1932 on a pure clover sward under temperature conditions which inhibited both growth and nitrification in the soil, showed that within a week of applying ammonium sulphate (646 gm. nitrogen per 1,000 sq. ft.), the nitrogen content of the clover had been increased significantly by 7.3 per cent.

Reviewing the evidence so far brought forward, it is seen that the slower rate of reduction in the clover content following upon the addition of sucrose with ammonium sulphate, cannot be explained satisfactorily merely by differences in the degree of competition between the grasses and the clover. The botanical changes are in agreement with the hypothesis that the diminution of clover is primarily connected with ammonium-ion poisoning as a result of a low carbohydrate reserve. The lack of response to nitrogenous manuring also indicates that carbohydrate rather than nitrogen is limiting growth. Sucrose can diminish the toxic effect of ammonium sulphate either, indirectly, after absorption by the roots of the clover, or by, directly, reducing the uptake of ammonium ions. It has been shown that the addition of sucrose together with ammonium sulphate leads to a reduction in the nitrogen content of the herbage and to a smaller absorption of nitrogen from the soil. In 1932, dressings of ammonium sulphate alone and of ammonium sulphate and sucrose, which gave yields

and nitrogen contents of the same order, produced different botanical changes. These differences suggest that sucrose was absorbed by the clover; but the evidence for this is not conclusive, since, although the nitrogen contents of the mixed herbage were the same in the two cases, both the proportions and the nitrogen contents of clover and the grasses may have been different with the two treatments. Lack of proof that sucrose is absorbed direct does not, however, invalidate the hypothesis put forward, since sucrose can also act by reducing the amount of ammonium ions taken up from the soil.

In addition to the main results of the experiment a comparison of the ammonia and nitrate fluctuations in the soil with the uptake of nitrogen by the turf has indicated that the added nitrogen has disappeared from the available (nitrate and ammonia) nitrogen of the soil to an extent which cannot be accounted for either by leaching or by uptake by the plant.

It seems highly probable that under grass, where the organic matter content is high, the growth of the soil microflora is controlled by lack of available nitrogenous material. It would seem likely, therefore, that the disappearance of the added nitrogen is due to assimilation by the microflora. Such a conclusion is in agreement with the disappearance of the nitrate nitrogen after the first calcium nitrate dressing. It might, however, be expected that the nitrogen thus assimilated would eventually be broken down again to an organic form. It is possible that the sudden marked increases in the ammonia and nitrate concentration (vide Figs. 11 and 13) were due to the release of nitrogen due to the death and decay of some portion of the microflora. Investigations (to be published elsewhere) carried out previously have indicated that this absorption and release of nitrogen may account in part for some of the losses of added nitrogen. That free nitrogen may be released either from the plant or from the soil is, however, also possible.

#### SUMMARY.

In a further study of the effect of nitrogenous compounds on the botanical composition of closely cut turf, additional evidence is presented to show that the reduction of the clover (*Trifolium repens*) brought about by ammonium sulphate is due to a toxic action of the absorbed ammonium ions.

By experiments during the years 1930-32 it has been demonstrated that weekly or fortnightly applications of ammonium sulphate at the rate of 278.5 gm. nitrogen per 1,000 sq. ft. (3 lb.  $(\text{NH}_4)_2\text{SO}_4$ /1,000 sq. ft.) led to a greater reduction in the content of *T. repens* than applications of sodium nitrate, calcium nitrate or urea, given at equivalent nitrogen rates. Ammonium nitrate in 1931 was intermediate in its effect between ammonium sulphate and calcium nitrate. In 1932 applications of ammonium

sulphate at two-thirds the standard rate still brought about a reduction of the clover.

Adding sucrose (4,540 gm. (10 lb.) per 1,000 sq. ft.) with each application of ammonium sulphate reduced the content of clover at a slower rate than did ammonium sulphate alone. Sucrose applied alone had no effect; sucrose added to either calcium or sodium nitrate had little effect on their action. If the reduction of the clover is due to a toxic action of the ammonium ion one would expect the addition of sucrose to ammonium sulphate to slow the rate of diminution, for the absorption of sucrose with the ammonium ions should lower the drain on the carbohydrate reserves resulting from the formation of amide compounds.

The view might be advanced that as sucrose is a readily available source of carbon its application leads to a large increase in the soil microflora which would thus absorb a considerable proportion of the added nitrogen. In consequence of the decreased availability of the nitrogen the intensity of competition between the grasses and the clover would be diminished. Experiments, however, on frequently cut plots showed that sucrose alone had little effect on the growth of the turf. Sucrose added to either ammonium sulphate, urea, or calcium nitrate brought about only a small reduction in the growth resulting from the nitrogenous manure alone.

The data from these experiments appear to rule out the possibility that the effect of sucrose is to decrease the intensity of competition. If such were its effect the addition of sucrose should bring about similar botanical changes whatever the nitrogenous fertilizer used, since the depression in growth was of the same order in each case. The botanical changes do not, however, show such an agreement.

In addition to investigating the botanical changes the uptake of nitrogen by the sward was also studied. Measurement of both the amount of nitrogen removed in the herbage and of the ammonia- and nitrate-nitrogen content of the soil showed that the whole of the added nitrogen could not be accounted by that in the plant or in the soil solution. From a study both of the fluctuations in the yield of weekly-cut plots and the weekly changes in the inorganic nitrogen content of the soil, it appears that much of the nitrogen added to turf is immediately absorbed by soil micro-organisms; subsequently, some of this nitrogen may again be released and become available to the plant.

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# Effect of Elliptically-polarized Light on the Formation of Carbohydrates in Leaves.

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With two Figures in the Text.

## INTRODUCTION.

THE synthesis of asymmetric compounds showing optical activity from optically inactive substances has always been a problem of interest to the chemist, but repeated attempts to effect a direct asymmetric synthesis by means of physically asymmetric influences have not so far met with success. There has been a large amount of work on the influence of circularly-polarized radiations on chemical reactions and a good review of the work done is given by Dhar (5).

The effect of plane-polarized radiation on biological processes has been a subject of investigation and the work done by various workers has already been reviewed by Dastur and Asana (2).

Sunlight near the sea surface is plane, elliptically, and circularly-polarized due to reflection and refraction at the sea surface. It is also believed that the synthesis of asymmetrical compounds first occurred in the vegetable organisms living in water. The synthesis of asymmetric compounds in plants occurs by the process of photosynthesis, therefore a study of the effect of plane-polarized light on the formation of carbohydrates in leaves of land-plants was undertaken by Dastur and Asana (2), but the results were negative. It was considered of interest to extend the work, and study the effect of elliptically-polarized radiations on the process of photosynthesis, since the effect of elliptically-polarized light on biological phenomena has not hitherto been a subject of investigation.

From a physical point of view, plane, elliptical and circularly-polarized

states of light, could be taken as modifications of one particular state—elliptical polarization. The latter is the more general, and the circular state is only a particular condition of the elliptical one, where the two axes of the ellipse happen to be equal. Similarly, plane polarization is also a condition of the elliptical state when one of the axes of the ellipse is reduced to an infinitely small length and consequently the light practically vibrates in one plane. The plane-polarized light could thus be taken as elliptically-polarized light with an infinitely eccentric ellipse. So, the elliptical state of polarization seems to be the most general and important, and it is therefore of interest to study the effect of this on the formation of carbohydrates in leaves.

#### INVESTIGATION.

##### *Method of Obtaining a Beam of Elliptically-polarized Light.*

The chief difficulty was found in devising a suitable apparatus to obtain a sufficiently large and intense beam of elliptically-polarized light. For physical experiments the elliptically and circularly-polarized lights are obtained by means of quarter-wave plates, but the elliptically-polarized light obtained by means of such plates did not serve our purpose, as only monochromatic light could be used, and it is well known that monochromatic light is not suitable for normal photosynthesis. Also the quarter-wave plates on the market are generally cut to suit a particular wave-length, generally the sodium light, and therefore were of no value for this investigation. There are other methods of obtaining elliptically-polarized light, such as the method of double reflection or refraction through a doubly-refracting crystal, but they are more or less of theoretical interest. The following method was employed to obtain a broad and intense beam of elliptically-polarized light, and a special apparatus was devised for the purpose.

Normal light when reflected from a glass surface at the Brewsterian angle of about  $57^\circ$  undergoes plane polarization. If such a reflected plane-polarized beam is again reflected by a metal mirror the emergent beam is elliptically polarized (R. Wood (6)), as all metallic surfaces are doubly refracting. In the case of silver the ellipticity obtained, almost approaches a circle.

The difficulty experienced by Dastur and Asana (2), in obtaining a beam of sufficient intensity was met here also. Reflections from the glass surface and the metal mirror involve a large loss of light. It is therefore very necessary to start with a powerful source so as to obtain a beam of the high intensity required for the experiments.

The same flood-light lamp used in the experiments of Dastur and Asana (2) was utilized as the source of light. It was placed slightly inclined to the horizontal so that its beam was incident on a pile of glass plates at an

angle of  $57^{\circ}$ . The glass plates were held in a wooden frame which could be rotated about a horizontal axis. The reflection becomes more and more efficient as the number of plates is increased and incidentally, the amount

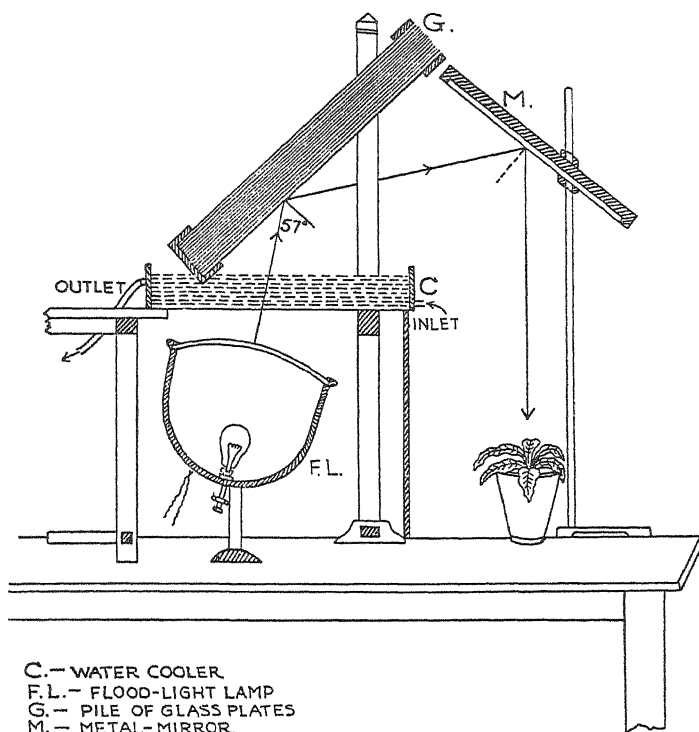


FIG. 1. A diagrammatic sketch of the apparatus for obtaining a beam of elliptically-polarized light.

of polarization also increases. In these experiments a pile of twenty-three glass plates was used giving about 90 per cent. of reflected plane-polarized radiation. The plane-polarized light was then reflected from a metallic surface. A metal mirror, to be described later, was placed in a suitable position in the path of the plane-polarized beam and the reflected beam from the metal mirror was elliptically polarized. The lamp, the pile of twenty-three glass plates and the metal mirror, were so arranged that a downward directed vertical beam of elliptically-polarized light was obtained (Fig. 1).

The task of selection of a metal mirror which would serve the purpose was difficult. Ready-made mirrors of sufficiently large size, are not available and the cost of their special manufacture is prohibitive. For a sufficiently broad beam a mirror of at least 24 in.  $\times$  24 in. was needed. It was therefore decided to use a highly polished metallic surface plated with silver and to determine if it would give elliptically-polarized light. A small

piece of copper plate, used for copper-plate printing, was electroplated with a thick coating of silver and the silver surface specially polished. This plate was then tested by means of a Babinet's compensator and it was found that it gave elliptical polarization of the plane-polarized light incident upon it. A highly polished copper plate of dimensions 24 in.  $\times$  24 in. was then similarly electroplated with a thick coating of silver and was specially polished. Before electroplating the copper plate was tested for uniformity of planeness. The silvered copper plate was then framed and was held by means of clamps in the proper position for obtaining elliptically-polarized light. For efficient working it is necessary that dust particles should not be allowed to settle on it and it should not be touched by hand. It should be kept covered by tissue paper and should be protected from fumes and gases. The vicinity of a chemical laboratory greatly interferes with the efficiency of the surface as all kinds of gases react with the metal and dull the surface. It was found necessary to replate the copper plate once during the progress of the work. The plate was always kept covered with a dark cloth when not in use. The heat rays were cut off by means of a water cooler (Fig. 2) with constantly circulating water which was placed between the lamp and the pile of glass plates held in the wooden frame. The water had to be circulated very rapidly in order to avoid the possibility of heat rays interfering with the intensity measurements.

It was also necessary to make control experiments with non-polarized light of the same intensity to study the differences in the carbohydrate contents of the leaves, exposed to elliptically-polarized light. For control experiments a 1,500 watt Phillips gas-filled lamp with an ordinary disc reflector was used.

The important factor in these experiments is the intensity of the light incident on the plants. The total intensity in the control experiments must be equal to the total intensity of the elliptically-polarized light. In all the experiments the total intensities were made equal by means of a micro-thermopile in conjunction with a D'Arsonval galvanometer. The total intensities of light used in each set of experiments were determined in terms of galvanometric deflexions and they are given in the tables of the results of carbohydrate analysis. The galvanometric deflexion varied from 13.5 cm. to 22.5 cm. in different sets of experiments but for the same set of experiments with polarized and normal light it was always the same.

#### CONSTANTS OF ELLIPTICAL POLARIZATION.

An accurate determination of these constants was carried out by means of the Babinet's compensator. The following procedure was adopted.

Before using the compensator, the readings of the micrometer screw were evaluated in terms of the wave-length of the light in question, in this

case it was heterogeneous light. This is done by allowing plane-polarized light to enter the instrument so that the plane of polarization makes an angle of approximately  $45^\circ$  with the axes of the two wedges. In this

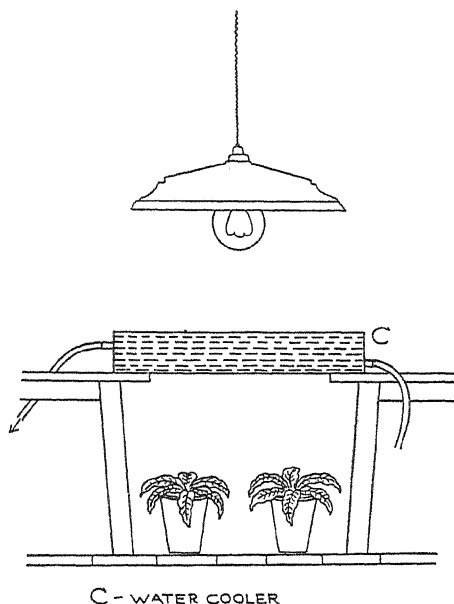


FIG. 2. A diagrammatic sketch of the apparatus for obtaining a normal beam of light.

position the fringes produced are most distinct. The moveable wedge is then adjusted with the micrometer screw until one of the fringes is on the cross-wire and the reading on the micrometer screw is then noted. The screw is then turned until the next band is on the cross-wire and the reading again noted. The difference corresponds to a phase difference of one wave-length. In this experiment this corresponds to  $2b = 1.937$  mm. After this the instrument is so adjusted that with plane-polarized light the central dark band corresponded with the cross-wire in the instrument. Elliptically-polarized light is then substituted and a shift in the position of the dark line is observed. At this band, the difference existing between the component vibrations has been neutralized by that introduced by the elliptical polarizer. This phase difference is consequently determined by turning the micrometer screw till the central dark band is brought under the wire. If the displacement be  $X$  we have,

$$\frac{a-\beta}{\pi} = \frac{X}{b} \text{ or, } a-\beta = \pi \frac{X}{b}$$

In our experiment,  $X = 0.51$  mms.

$$\therefore a-\beta = \pi \frac{0.51}{0.968} = 0.53\pi = \text{Phase difference.}$$

The ratio of the axes could not be accurately determined as the fringes became very faint on turning the compensator. It was therefore omitted. The phase difference, however, was enough to show that the light was elliptically polarized.

An attempt was first made to study the formation of starch in starved leaves of potted plants under the elliptically polarized and normal lights of equal intensities, but the amount of starch formed as revealed by the iodine test was insignificant after even twelve hours' exposure. It was therefore necessary to determine quantitatively the different carbohydrates produced in leaves exposed to the two lights.

The method of extracting the carbohydrates from leaves worked out by Davis, Daish, and Sawyer (4) was adopted in the main in this investigation. The method used for estimating the sugars and starches was the revised method of Calvert (1 and 2) improved by Dastur and Samant (3).

The following plants were employed :

(1) *Nicotiana Tabacum*. (2) *Tropaeolum majus*. (3) *Raphanus sativus*. (4) *Allium Cepa*.

The precautions mentioned above were taken in the selection of plants for experimentation. About twelve plants were needed for each set of experiments. The plants were kept in the dark for about thirty-six to forty-eight hours. The leaves and petioles of four plants were taken directly for carbohydrate analysis and four plants were exposed to non-polarized light and the remaining four to the polarized beam of light. After six hours of exposure, the leaves and petioles of the exposed plants were analysed separately, according to the method described above. All the weights are given on the basis of fresh weight and the results of the carbohydrate analysis are expressed as percentages of the fresh weights of the leaves. The following tables give the results.

In Table I the results of the carbohydrate contents of the leaves of *Nicotiana Tabacum*, exposed to two lights and of the leaves from the dark are given. Similarly the Tables II to IV give the results of the carbohydrate contents of the leaves of *Tropaeolum majus*, of *Raphanus sativus*, and of *Allium Cepa* respectively.

In all, fourteen sets of experiments with four different species were performed and the number of carbohydrate analyses of leaves was forty-two.

A study of the results shows that the carbohydrate contents of the leaves exposed to elliptically-polarized light, are less than the carbohydrate contents of the leaves exposed to non-polarized light of equal intensity. Only in one instance is the carbohydrate content of the leaves of *N. Tabacum* (Table I, Experiment No. 2) exposed to polarized light higher than that of the leaves of the same species exposed to normal light.

Before any conclusions are drawn it is necessary to determine whether

TABLE I.

*The Carbohydrate Content (gm. per 100 gm. Fresh Weight) of the Leaves of Plants Exposed to Elliptically Polarized and Normal Lights.*

*Nicotiana Tabacum.*

(1) 13. I. 33-14. I. 33.

Total intensity = 17 cm. deflexion.

	Reducing sugars as hexoses.	Sucrose as hexoses.	Starch as hexoses.	Total carbohydrates as hexoses.
Non-polarized	Nil	0.0844	0.0094	0.0938
E. polarized	Nil	0.0477	0.0111	0.0588

(2) 12. I. 33-13. I. 33.

Total intensity = 17.5 cm.

	Reducing sugars as hexoses.	Sucrose as hexoses.	Starch as hexoses.	Total carbohydrates as hexoses.
Non-polarized	Nil	0.0296	0.0025	0.0321
E. polarized	Nil	0.0507	0.0024	0.0531

(3) 16. 3. 33-17. 3. 33.

Total intensity = 19.5 cm.

	Reducing sugars as hexoses.	Sucrose as hexoses.	Starch as hexoses.	Total carbohydrates as hexoses.
Non-polarized	Nil	0.0646	Nil	0.0646
E. polarized	Nil	0.0481	Nil	0.0481

(4) 3. 4. 33-4. 4. 33.

Total intensity = 19.5 cm.

	Reducing sugars as hexoses.	Sucrose as hexoses.	Starch as hexoses.	Total carbohydrates as hexoses.
Non-polarized	Nil	0.0158	0.0027	0.0185
E. polarized	Nil	0.0122	0.0025	0.0147

TABLE II.

*Tropaeolum majus.*

(1) 21. 2. 33-22. 2. 32.

Total intensity = 19.5 cm.

	Reducing sugars as hexoses in gm.	Sucrose as hexoses in gm.	Starch as hexoses in gm.	Total carbohydrates as hexoses in gm.
Non-polarized	Negligible	0.0486	0.0050	0.0536
E. polarized	"	0.0250	0.0030	0.0280

(2) 1. 3. 33-2. 3. 33.

Total intensity = 22.5 cm.

	Reducing sugars as hexoses in gm.	Sucrose as hexoses in gm.	Starch as hexoses in gm.	Total carbohydrates as hexoses in gm.
Non-polarized	0.0190	0.5710	Negligible	0.5900
E. polarized	0.0140	0.4027	"	0.4167

(3) 10. 3. 33-11. 3. 33.

Total intensity = 21 cm.

	Reducing sugars as hexoses in gm.	Sucrose as hexoses in gm.	Starch as hexoses in gm.	Total carbohydrates as hexoses in gm.
Non-polarized	Nil	0.1583	0.0063	0.1646
E. polarized	Nil	0.1095	0.0078	0.1173

TABLE III.

*Raphanus sativus.*

(1) 26. 9. 32–27. 9. 32.

Total intensity = 13 cm.

	Reducing sugars as hexoses in gm.	Sucrose as hexoses in gm.	Starch as hexoses in gm.	Total carbohydrates as hexoses in gm.
Non-polarized	0.0166	0.0711	0.0114	0.0991
E. polarized	Nil	0.0698	0.0145	0.0843

(2) 1. 10. 32–2. 10. 32.

Total intensity = 14.5 cm.

	Reducing sugars as hexoses in gm.	Sucrose as hexoses in gm.	Starch as hexoses in gm.	Total carbohydrates as hexoses in gm.
Non-polarized	Nil	0.0441	0.0072	0.0513
E. polarized	Nil	0.0242	0.0049	0.0291

(3) 12. 10. 32–13. 10. 32.

Total intensity = 13.5 cm.

	Reducing sugars as hexoses in gm.	Sucrose as hexoses in gm.	Starch as hexoses in gm.	Total carbohydrates as hexoses in gm.
Non-polarized	Nil	0.0234	0.0103	0.0337
E. polarized	Nil	Negligible	0.0080	0.0080

TABLE IV.

*Allium Cepa.*

(1) 8. 2. 33–9. 2. 33.

Total intensity = 17.5 cm.

	Reducing sugars as hexoses in gm.	Sucrose as hexoses in gm.	Total carbohydrates as hexoses in gm.
Non-polarized	Nil	0.0292	0.0292
E. polarized	0.0050	0.0257	0.0307

(2) 25. 3. 33–26. 3. 33.

Total intensity = 18.5 cm.

	Reducing sugars as hexoses in gm.	Sucrose as hexoses in gm.	Total carbohydrates as hexoses in gm.
Non-polarized	0.0175	0.1205	0.1380
E. polarized	0.0228	0.0767	0.0995

(3) 8. 4. 33–9. 4. 33.

Total intensity = 19.5 cm.

	Reducing sugars as hexoses in gm.	Sucrose as hexoses in gm.	Total carbohydrates as hexoses in gm.
Non-polarized	Nil	0.0657	0.0657
E. polarized	Nil	0.0265	0.0265

(4) 13. 4. 33–14. 4. 33.

Total intensity = 19.5 cm.

	Reducing sugars as hexoses in gm.	Sucrose as hexoses in gm.	Total carbohydrates as hexoses in gm.
Non-polarized	0.0087	0.0480	0.0567
E. polarized	Nil	0.0500	0.0500



the differences in the carbohydrate contents of the leaves exposed to non-polarized and elliptically-polarized light are significant. The results were tested by Fischer's method of 't'. In these fourteen experiments the value of  $P$  was found to be 0.03. The differences between the carbohydrate contents of the leaves exposed to normal and elliptically-polarized lights are therefore significant.

#### CONCLUSIONS.

This investigation clearly shows that the elliptically-polarized light has no accelerating influence on the formation of carbohydrates in leaves. On the contrary the elliptically-polarized light unlike the plane-polarized (see Dastur and Asana (3)) has a depressing effect on the process, as, the values of carbohydrate contents of the leaves are definitely lower than those of the leaves exposed to normal light. It is difficult in the present state of knowledge to assign any reason for the lower values of the carbohydrate contents of the leaves in elliptically-polarized light. The results clearly show that the influence of elliptically-polarized radiations on the formation of carbohydrates is different from that of the plane-polarized radiations, and therefore the plane of vibrations of light may have something to do with the synthesis of asymmetric compounds.

If a method can be devised by means of which the total optical activity of the substances produced in leaves exposed to polarized and normal light can be accurately measured, it would be possible to arrive at a more definite conclusion concerning the effect of differently polarized radiations on the synthesis of asymmetric compounds. As the leaf contains such a heterogeneous mixture of very complex substances in minute quantities many difficulties will have to be overcome before such a method can be devised.

#### SUMMARY.

The effect of elliptically-polarized light on the formation of carbohydrates in leaves was determined.

A special apparatus was devised to obtain elliptically-polarized light; this was done by re-reflecting a beam of plane-polarized light from a metal mirror as reflected by a pile of glass plates kept at the Brewsterian angle to a beam of parallel rays from a powerful flood-light lamp. The resultant beam was elliptically polarized and broad enough to illuminate four plants at a time. Potted plants kept previously in the dark for thirty-six hours were exposed side by side for six hours to the elliptically-polarized beam and a normal beam of equal intensity.

Analysis of the leaves showed that the carbohydrate content of the leaves in elliptically-polarized light was less than that of the leaves exposed to non-polarized light.

The results have been examined statistically and the differences are found to be significant. This indicates a depressing or retarding effect of elliptically-polarized light on the formation of carbohydrates in leaves.

We have to thank the University of Bombay for a grant of Rs. 350 in 1931-2 towards the expenses of this investigation.

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# The Effect of Ionized Air on the Respiration of Green Plants.

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With four Figures in the Text.

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## 1. INTRODUCTION.

IN 1927 positive increases in respiration under the influence of ionized air were obtained by Whimster (5), using pelargonium leaves, and by Middleton (4), working with barley seedlings. Later, De Boer (2), using a very wide range of ionization values, was unable to obtain any effect on the respiration of fungi.

In view of these results, further work on this subject was considered necessary, and the present investigation was undertaken.

## 2. EXPERIMENTAL TECHNIQUE.

### *Material.*

Experience having been obtained with barley seedlings and leaves of pelargonium, it was decided to employ both.

The barley was a pure line (var. Goldthorpe), originally obtained from the Cereal Station of the Department of Agriculture and Technical

Instruction for Ireland and grown at Rothamsted in 1930. Approximately 100–200 seeds were taken each day, placed in a large Petri dish lined with moist filter paper and allowed to germinate at 17° C. After 3–4 days

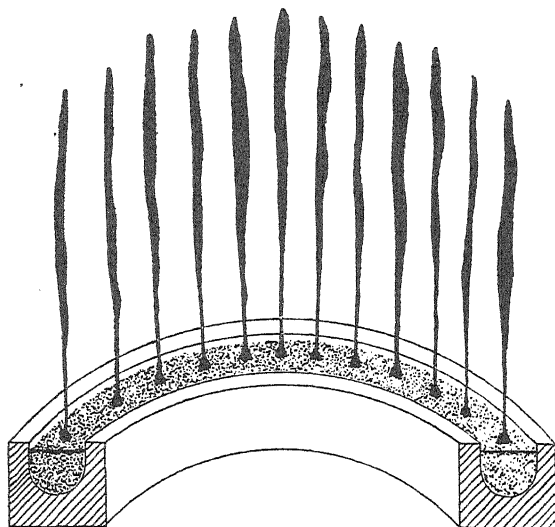


FIG. 1. Section of wax ring, showing method of growing barley seedlings.

a number of the germinated seeds, selected for uniformity and freedom from fungal infection, were planted out in a wax ring containing moist silver sand (Fig. 1). The average number of seeds in each ring was about fifty. The ring containing the seeds was now placed under artificial illumination at a temperature between 20° and 22° C. and watered daily. The experiment was started when the seedlings had reached an average height of 10 cm.; either the fourth or fifth day after planting. The illumination provided came from a water-screened Osram gas-filled lamp of 750 watts, placed at a distance of 40 cm. from the plants.

For the pelargonium experiments, leaves of *P. zonale* (var. Paul Crampel) were employed. The plants were grown at Chelsea Physic Garden, cut leaves, selected as far as possible for size and age, being brought to the laboratory each morning. The petioles were now cut to the required length, the leaves placed in a glass stand, and allowed to assimilate for twenty-four hours under the same illumination as employed for the barley. Before use they were transferred to a special glass ring stand (Fig. 2), designed to maintain the leaves in their correct positions in the respiration chamber.

#### *Apparatus.*

The respiration apparatus employed was the same for all the experi-

mental sets except the last, in which the original apparatus as described by Whimster was used. The new apparatus had a metal respiration chamber, and the circuits were so arranged that either a continuous current of air or a closed system experiment could be conducted. It is shown diagrammatically in Figure 3.

Starting from the left of the diagram, atmospheric air from outside the laboratory passes through the dust trap  $A_1$ , and by way of the two-way taps  $T_1$  and  $T_2$ , to the spiral scrubber  $S$  containing fifty per cent.  $\text{NaOH}$ . Following this is a trap  $B_1$  and two towers, the first  $A_2$  containing calcium chloride to protect the soda lime in  $A_3$ . From here, the air now completely freed from  $\text{CO}_2$ , passes by way of the gas wash-bottle  $B_2$  to the taps leading to the various circuits.  $B_2$  was filled with normal  $\text{Ba}(\text{OH})_2$ , the level being kept constant by periodic additions of distilled water, and served both as a test bubbler and to maintain a fairly constant degree of saturation in the air stream. Owing to the slight negative pressure at this point the tap  $T_3$  was inserted to prevent 'sucking back' of the  $\text{Ba}(\text{OH})_2$  on the chamber being opened.

Of the three circuits meeting after  $B_2$ , the first via the tap  $T_4$ , formed a complete by-pass to the whole chamber and circulation system. The second, controlled by the three-way tap  $T_5$ , formed part of the circulation system, while the third led direct to chamber  $C$ , by way of the condensation water trap  $K$ . A manometer  $M$  to record the pressure in the chamber was inserted in this last circuit.

The chamber was cylindrical in shape with a volume of about 3 litres. It was solidly constructed of brass with a gun-metal flange and had a gun-metal lid. This was held in place by six bolts; an air-tight connexion being ensured by means of a vaselined lead washer. The ionizing agent—polonium deposited on copper foil—was symmetrically disposed around a brass rod passing through a rubber stopper in the lid. When not in use the polonium could be drawn up into a small compartment which was then closed by a brass cone attached to the bottom of the carrying rod. In this position there was no ionization of the air in the chamber proper.

The whole chamber was immersed in a water-bath, the temperature being kept constant within  $0.2^\circ \text{C}$ . by means of the mercury thermostat  $Th$  and the heating lamp  $H$ . The two motor-driven stirrers used are not shown in the diagram. Incidentally it may be added that both the water-bath and chamber were provided with circular celluloid windows in the bottom to permit of X-rays being employed as the ionizing agent.

Passing from the chamber to the three-way tap  $T_7$ , connexion could either be made to the absorption bubblers  $D_1 D_2$  by way of the trap  $B_3$ , or to the circulating pump  $P$ . This was a mercury pump of the Blackman-Bolas type, but was fitted with an interrupter  $I$  of new design. It was provided with a gold-leaf trap  $L$ .

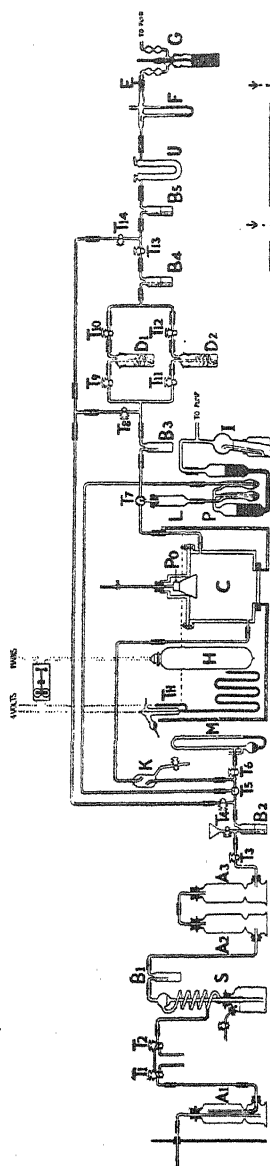


FIG. 3. Diagram of apparatus. For explanation see text.

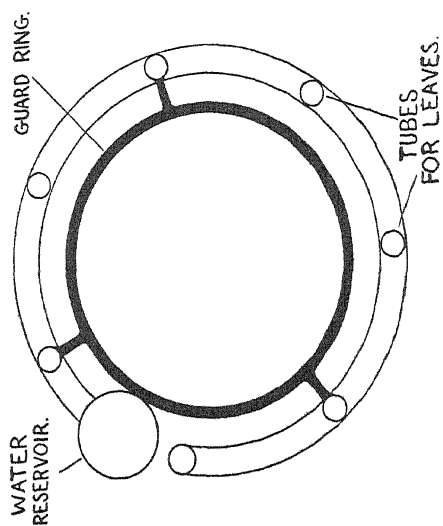


FIG. 2. Plan of ring stand for holding pelargonium leaves.

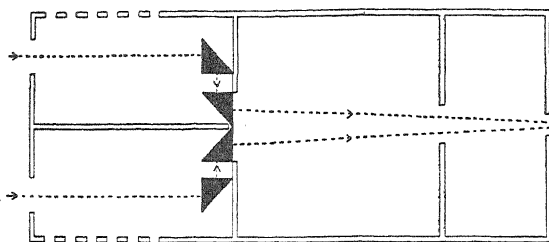


FIG. 4. Diagram of comparator, showing prisms and position of slots for the coloured glasses.

The two absorption bubblers used were of the very efficient type provided with an internal spiral, and were so arranged that either of them could be isolated by means of the taps  $T_{9-12}$ , and their contents replaced without interference with the other.

Following the bubblers was a small wash-bottle  $B_4$ , containing  $Ba(OH)_2$ , and serving to indicate the degree of absorption of the carbon dioxide by the two bubblers; incomplete absorption was never observed.

The whole system of absorption bubblers could be by-passed by way of the taps  $T_8$  and  $T_{14}$ , a tap  $T_{13}$  being fitted in the position shown, to prevent contamination of the test baryta in  $B_4$  by the  $CO_2$  in the gas stream.

Flow regulation was controlled by adjusting the screw clip E, and was kept at six litres per hour by means of the Venturi flow-meter F, containing mercury. Owing to the clogging of the flow-meter jet, it was found necessary to dry and clean the air before it passed through. This was accomplished by the gas wash-bottle  $B_5$ , containing concentrated  $H_2SO_4$  and the U tube U, one limb of which contained  $CaCl_2$ , while the other was packed with cotton wool.

Gas flow was maintained by means of an ordinary water pump, the Mariotte bottle G giving a constant 'head' of negative pressure. Although this was fairly large, the negative pressure in the chamber never reached 2 cm. of mercury since the major part of the resistance in the system was on the pump side of the chamber.

All joints were 'glass to glass', the rubber tubing used being of the heavy pressure type, thoroughly impregnated with paraffin wax under reduced pressure. After fitting the rubber, the joints were painted with shellac.

Tests for leaks were made from time to time by leaving the whole system under a high negative pressure. The only trouble experienced was due to the wearing of the lead washer on the chamber. This only resulted in a small amount of water entering from the bath, and was easily remedied by fitting a new washer.

For the last set of experiments no departure was made from the apparatus as used by Whimster; a description is therefore unnecessary.

All experiments were carried out at  $25^\circ C$ .

### *Carbon dioxide estimation.*

In all experiments with the new apparatus this was carried out by absorption in sodium hydroxide and subsequent titration. The procedure was as follows: 50 c.c. of 0.2 N. NaOH was delivered from an automatic pipette into the bottom half of the spiral bubbler, and the top, already vaselined, rapidly replaced. After absorption of the respiratory  $CO_2$ , the

bubbler was very thoroughly washed out into a 250 c.c. graduated flask; 5 c.c. of 1.0 N.  $\text{BaCl}_2$  added, and the liquid made up to the mark with distilled water. All distilled water used was  $\text{CO}_2$  free.

It may be noted at this point that it was found necessary to calibrate all the graduated glassware used, some of the 250 c.c. flasks having errors of over 0.5 c.c.

After the precipitate of  $\text{BaCO}_3$  had settled, the liquid usually being allowed to stand overnight, the flask was attached to an automatic pipette and a measured portion of the clear  $\text{NaOH}$  delivered into the titrating flask. This operation was accomplished, under entirely  $\text{CO}_2$ -free conditions and without disturbing the precipitate by means of the pipette, designed in this laboratory by J. L. Armstrong and the author, and to be elsewhere described.

For the actual titration, a known quantity of approximately 0.04 N.  $\text{HCl}$  was run into the titrating flask from an automatic pipette, and the titration completed with 0.02 N.  $\text{HCl}$ , using phenolphthalein as indicator. During the whole of this process it was found advisable to keep the titrating flask closed by a thin rubber cap, pierced with a small hole for the burette. Two replicates were always titrated from each 250 c.c. flask.

Since, in the method employed, only the burette readings for the weaker acid were taken, the  $\text{CO}_2$  value was obtained by subtraction of these figures from those obtained with a control titration.

In the final set of experiments with the Whimster apparatus, in which the indicator method as elaborated by Bolas (1) was used, the same procedure as described by Whimster (5 p. 363) for determining respiration rates was followed in its entirety. One small change was however made in the apparatus for colour matching, the portion of the box containing the slots for the coloured glasses being replaced by a simple form of comparator. This rendered matching very much easier, the two 'colour fields' being brought side by side by means of totally reflecting glass prisms. A diagram showing the optical system is given in Figure 4.

### *Ionization.*

As in previous work, polonium was again found to be a very satisfactory source of ionization, and was employed in all the experiments. The polonium, deposited on a strip of copper foil, was obtained from the Institut du Radium, Paris.

The method of using the polonium in the new chamber has already been described; when in position for ionizing the air it was suspended centrally, about 10 cm. from the bottom of the chamber. Both the barley seedlings and the pelargonium leaves were provided with a circular guard ring, approximately 10.5 cm. in diameter. This was considered necessary in order to prevent any possibility of the plants coming within the range of



the  $\alpha$  particles from the polonium. One of these rings is shown in Figure 2.

In the last set of experiments the polonium was mounted and used in the same way as described by Whimster. It was, however, a different piece from that used in the other sets.

*Measurement of degree of ionization.*

The determinations were made in the usual way by measuring the saturation current between two plates in the position normally occupied by the seedlings or leaves. One plate was connected to a Dolazalek electrometer, in parallel with a precision air condenser of  $.001 \mu f$ . The other plate was charged by means of a rotary converter and dry batteries giving a total E.M.F. of about 1,100 volts, this being more than sufficient to give a saturation current.

Using this method several measurements of the saturation current were made, of which the most accurate are given in Table I.

TABLE I.

*Ionisation Determinations.*

Date.	Sets.	Volume of air between plates (cm. <sup>3</sup> ).	Total capacity ( $\mu f$ ).	Saturation current (amps.).	Degree of ionization (Normal air = 1).
27. 10. 31	A-E	4	0.00106	$8.8 \times 10^{-11}$	$1.4 \times 10^5$
13. 2. 33	F	70	0.00109	$30.5 \times 10^{-11}$	$2.8 \times 10^4$

As the half-life period of polonium is 136 days (4), and following Wilson (7) in taking normal air as containing 1,000 ions per c.c., one can calculate the degree of ionization in terms of normal air for each set of experiments. These figures are given with their respective experimental results.

Following Whimster, Middleton, and De Boer, all measurements were made in still air dried with calcium chloride.

### 3. EXPERIMENTAL RESULTS.

*Barley Seedlings.*

*Set A.*

The ring of seedlings was placed in the chamber overnight with the gas stream flowing straight through the apparatus.

The following morning, after allowing twelve hours for respiration to become steady, readings were begun, the respiratory  $CO_2$  being absorbed continuously for five successive two-hour periods. In the experiments with ionized air the polonium was applied during the second and fourth periods.

TABLE II.

*Barley Seedlings. Set A.*

CONTROLS. April to July, 1931.

No.	Date.	Respiration in mg. CO <sub>2</sub> per 2-hour period.				
		1	2	3	4	5
1.	27.4	18.47	17.96	18.27	18.92	17.89
2.	29.5	15.83	16.51	16.63	16.68	17.02
3.	1.6	23.91	25.02	25.52	25.66	25.26
4.	3.6	22.74	23.59	23.79	23.75	24.10
5.	4.6	23.83	24.93	24.57	25.05	24.74
6.	16.6	22.31	21.91	22.32	22.64	22.58
7.	18.6	19.77	20.91	21.03	20.23	20.05
8.	13.7	13.42	14.26	13.99	14.21	14.38
9.	20.7	18.42	18.92	18.87	18.35	18.41
10.	23.7	18.99	19.20	18.79	18.23	18.15
Mean = 19.77		20.32	20.38	20.37	20.26	20.26
S. E. = $\pm 1.10$		$\pm 1.14$	$\pm 1.17$	$\pm 1.20$	$\pm 1.17$	$\pm 1.17$

*Experimental. April to July, 1931.*

IONIZATION. 300,000 times normal.

No.	Date.	Respiration in mg. CO <sub>2</sub> per 2-hour period.				
		1	2*	3	4*	5
1.	23.4	19.77	20.36	20.17	20.74	19.87
2.	24.4	20.89	21.88	21.72	21.00	20.22
3.	28.4	17.09	17.34	16.63	17.11	16.77
4.	30.4	19.46	19.80	19.17	19.44	18.92
5.	1.5	22.62	23.38	22.58	24.28	24.53
6.	9.6	22.53	23.29	23.79	24.19	24.40
7.	17.6	23.95	24.19	23.10	24.28	23.88
8.	22.6	19.86	21.91	21.98	20.56	19.96
9.	16.7	13.90	14.75	14.85	15.56	15.73
10.	24.7	17.35	18.06	17.06	17.89	17.11
Mean = 19.74		20.50	20.11	20.51	20.14	20.14
S. E. = $\pm 0.95$		$\pm 0.96$	$\pm 0.97$	$\pm 0.98$	$\pm 1.02$	$\pm 1.02$

TABLE III.

*Barley Seedlings. Set B.*

CONTROLS. November to December, 1931.

No.	Date.	Respiration in mg. CO <sub>2</sub> per 2-hour period.				
		1	2	3	4	5
1.	5.11	19.77	20.01	20.17	19.91	19.79
2.	13.11	17.52	18.20	18.13	17.80	17.32
3.	19.11	19.20	19.23	19.16	19.39	18.66
4.	3.12	17.30	16.64	16.80	16.51	16.80
5.	8.12	17.61	17.42	17.58	17.06	17.11
Mean = 18.28		18.30	18.37	18.13	17.94	17.94
S. E. = $\pm 0.50$		$\pm 0.61$	$\pm 0.59$	$\pm 0.66$	$\pm 0.56$	$\pm 0.56$

TABLE III (continued).  
*Experimental. November to December 1931.*

IONIZATION. 125,000 times normal.						
No.	Date.	Respiration in mg. CO <sub>2</sub> per 2-hour period.				
		1	2*	3	4*	5
1.	6.11	18.65	19.06	18.87	18.75	18.41
2.	17.11	18.56	18.54	19.04	18.61	18.66
3.	20.11	15.97	17.54	17.58	17.37	17.92
4.	27.11	15.49	16.64	17.40	17.75	17.75
5.	1.12	18.39	18.80	18.18	18.01	17.97
	Mean =	17.41	18.12	18.21	18.10	18.14
	S. E. =	± 0.69	± 0.45	± 0.33	± 0.26	± 0.17

TABLE IV.  
*Pelargonium Leaves. Set C.*

CONTROLS. January to February, 1932.				
No.	Date.	Respiration in mg. CO <sub>2</sub> per 100 cm. <sup>2</sup> leaf area per 2-hour period.		
		1	2	3
1.	27.1	1.70	1.58	1.47
2.	3.2	3.19	3.05	2.93
3.	9.2	2.53	2.44	2.24
4.	12.2	2.30	2.28	2.16
5.	17.2	2.92	2.76	2.67
	Mean =	2.53	2.42	2.29
	S. E. =	± 0.28	± 0.25	± 0.25

*Experimental. January to February 1932.*

IONIZATION. 85,000 times normal.				
No.	Date.	Respiration in mg. CO <sub>2</sub> per 100 cm. <sup>2</sup> leaf area per 2-hour period.		
		1	2*	3
1.	26.1	1.86	1.72	1.61
2.	2.2	2.64	2.44	2.34
3.	4.2	2.94	2.95	2.86
4.	10.2	2.52	2.34	2.30
5.	16.2	2.53	2.30	2.22
	Mean =	2.50	2.35	2.27
	S. E. =	± 0.18	± 0.20	± 0.20

The full experimental data for the control and ionized series are given in Table II.

#### *Set B.*

This was carried out in the same way as Set A, the only difference being in the smaller number of experiments. The experimental data are given in Table III.

*Pelargonium Leaves.**Set C.*

The ring stand containing the leaves was placed in the chamber with the gas stream flowing straight through the apparatus. After two hours, readings were begun, the respiratory  $\text{CO}_2$  being absorbed continuously for three successive two-hour periods. In the experiments with ionized air the polonium was applied during the second period.

*Sets D–F.*

The experimental data are given in Table IV.

The three following sets were designed to test whether there was any increased effect of ionized air, consequent upon the accumulation of respiratory carbon dioxide, which takes place in closed system experiments. It was also considered possible that mercury vapour from the Blackman-Bolas pump might have affected the results in the original Whimster experiments. These were therefore repeated in Set F.

*Set D.*

The ring stand containing the leaves was placed in the chamber and the gas stream allowed to flow straight through. After two hours taps  $T_5$  and  $T_7$  were turned so that the stream of  $\text{CO}_2$  free air was diverted through the circulating pump P, the chamber being isolated. After five minutes for clearing the pump, these taps were again turned so as to put the pump in circuit with the chamber, the gas stream being allowed to flow through the by-pass by opening tap  $T_4$ . After working as a closed system for three hours fifty-five minutes,  $T_4$  was closed and the pump put in communication with one of the absorption bubblers D, the chamber being isolated. After allowing five minutes for any  $\text{CO}_2$  in the pump to be absorbed, it was put out of circuit and the gas stream permitted to flow straight through and sweep out the chamber, one hour fifty-five minutes being allowed for absorption of the accumulated respiratory  $\text{CO}_2$ . The duration of the whole experiment was six hours, for which only one respiration reading was obtained.

In the experimental series the polonium was applied during the four hours in which the closed-system-working was in operation.

The respiration data, reduced to a two-hour basis, are given in Table V.

*Set E.*

This was similar in Set D, but was carried out at a different time of year. The respiration data are given in Table VI.

*Set F.*

This was a repetition of the respiration experiments of Whimster, the same procedure as described on page 363 of her paper being followed.

TABLE V.

*Pelargonium Leaves. Set D. March 1932.*

IONIZATION. 70,000 times normal.

Respiration in mg. CO<sub>2</sub> per 100 cm.<sup>2</sup> leaf area per 2 hours.

CONTROLS.	EXPERIMENTAL.
2.71	2.86
2.60	2.83
2.79	2.39
2.68	3.13
3.14	3.42
Mean = 2.78	2.93
S. E. = $\pm 0.09$	$\pm 0.17$

TABLE VI.

*Pelargonium Leaves. Set D. June 1932.*

IONIZATION. 45,000 times normal.

Respiration in mg. CO<sub>2</sub> per 100 cm.<sup>2</sup> leaf area per 2 hours.

CONTROLS.	EXPERIMENTAL.
3.93	4.80
4.06	3.73
4.05	4.05
4.06	4.22
4.83	4.55
Mean = 4.19	4.27
S. E. = $\pm 0.16$	$\pm 0.19$

TABLE VII.

*Pelargonium Leaves. Set F.*

Respiration in Closed System using Indicator Method.

CONTROLS. December, 1932 to January, 1933.

No.	Date.	Respiration in mg. CO <sub>2</sub> per 100 cm. <sup>2</sup> leaf area per 2-hour period.		
		1	2	3
1.	14.12	3.90	4.56	3.25
2.	15.12	4.48	4.48	3.58
3.	16.12	4.46	4.74	4.46
4.	20.12	4.15	4.43	4.98
5.	3.1	3.15	3.79	3.47
6.	4.1	3.88	3.45	3.45
7.	5.1	3.01	3.27	3.27
8.	6.1	2.83	2.47	3.25
9.	11.1	4.52	4.34	4.17
10.	12.1	3.66	3.82	3.99
	Mean = 3.80		3.94	3.79
	S. E. = $\pm 0.20$		$\pm 0.23$	$\pm 0.19$

TABLE VII (*continued*).*Experimental. January to February, 1933.*

IONIZATION. 30,000 times normal.

		1	2*	3
1.	17.1	4.09	4.46	4.28
2.	18.1	4.16	5.19	4.41
3.	19.1	2.90	2.73	3.22
4.	20.1	2.22	2.66	2.66
5.	25.1	2.78	3.02	3.62
6.	27.1	2.51	2.51	2.31
7.	31.1	2.22	2.41	2.66
8.	1.2	2.64	3.17	2.90
9.	2.2	3.17	2.82	3.00
10.	3.2	4.07	3.66	3.46
		Mean = 3.08	3.26	3.25
		S. E. = $\pm 0.24$	$\pm 0.29$	$\pm 0.15$

The experimental data for this set are given in Table VII.

Owing to the author being slightly colour-blind, the actual experimental work required for this set was carried out by Mr. J. J. Chinoy, to whom the author wishes to express his thanks for the very thorough way in which the work was accomplished.

TABLE VIII.

*Barley Seedlings. Set A.*

## CONTROLS.

Variance due to	Degrees of freedom.	Sums of squares.	Mean square.
Plants	9	596.3326	
Time	4	2.6281	
Residual	36	6.0262	0.1674
Total	49	604.9869	

## EXPERIMENTAL.

Variance due to	Degrees of freedom.	Sums of squares.	Mean square.
Plants	9	416.3176	
Time	4	4.0312	
Residual	36	12.8080	0.3558
Total	49	433.1568	

## (4) STATISTICAL TREATMENT OF RESULTS.

In Sets A, B, C, and F it was found possible to use the method of 'Analysis of variance': Accordingly, the actual application of this method will be described in detail for Set A.

Taking the data of Table II, the variance for each series can be

analysed as shown in Table VIII. Then assuming the best estimate of the standard error for the whole set to be obtainable from the residual variances of the two series, we have

$$\frac{\text{Sum of squares}}{\text{No. of degrees of freedom}} = \frac{6.0262 + 12.8080}{36 + 36}$$

giving a Mean Square for the whole set of 0.2616.

Now considering periods 1, 3, and 5 untreated, and periods 2 and 4 treated, we have

Total number in untreated periods = 60

Total number in treated periods = 40

whence

$$\begin{aligned}\text{Standard error} &= \sqrt{(\frac{1}{60} + \frac{1}{40}) 0.2616} \\ &= 0.1044\end{aligned}$$

Then calling the mean of the treated periods  $M_t$  and the mean of the untreated periods  $M_u$  we get

Experimental series  $M_t - M_u = 0.5052$

Control series  $M_t - M_u = 0.211$

If now the value for the control series be subtracted from the value for the experimental series, thus allowing for the shape of the respiration curve, we get the value 0.2942 which may be called the 'treatment difference' and to which the 't' test can be applied by dividing by the standard error giving  $t = 2.818$  where  $n = 98$ . This shows a probability of considerably more than 100 to 1 and is certainly highly significant.

Examined in the same way Set B gives

Experimental series  $M_t - M_u = 0.184$

Control series  $M_t - M_u = 0.022$

Differences 0.162

The standard error of the difference is 0.1219 where  $t = 1.329$  ( $n = 48$ ). This probability is little more than five to one and is not significant.

Similarly Sets C and F give 'treatment differences' of -0.043 and -0.0405 with standard errors of 0.0871 and 0.0918 respectively. The results are not significant; there is thus no evidence of any positive effect in either case.

In the case of Sets D and E it is only possible to make a direct comparison between the means of the experimental and control series. For this purpose the 't' test is as convenient as any and was therefore used, values of 't' equal to 0.768 and 0.338 being obtained for Sets D and E respectively. Both these values are quite non-significant, but there is some slight evidence for a positive effect in that the means of the experimental series are higher than those of the control series in both sets. It is, however, obvious that these differences will have to be very large to

become significant, since the only measure of variability available for comparison with the effect of treatment includes the variability of the plant material, which is known from the other experiments to be considerable. The standard error is thus much exaggerated. The increases due to treatment were actually five per cent. and 2 per cent., which are greater than that found in the one significant result with barley.

It is interesting to note that from the data already obtained a direct comparison of the accuracy of the indicator method of Set F and the continuous absorption method of Sets A–C can be made. Thus if we compare Set F with Set A, both having the same number of individual experiments, we have standard errors of 0.0918 for Set F and 0.1044 for Set A. Then since an increase equal to twice the standard error is necessary for significance we obtain the values 0.183 and 0.209. Treating these as absolute increases in respiration they come to six per cent. and one per cent. of the experimental values in Sets F and A respectively, showing that the indicator method requires an effect six times as great as that possible of detection with the continuous absorption method.

#### (5) DISCUSSION OF RESULTS.

Set A with barley seedlings shows a very significant increase in respiration under the influence of ionized air. The effect is very small, being an increase of only about 1.5 per cent. of the respiration rate. Set B shows a slight increase which, though not significant, is no doubt a real effect of the treatment.

Turning to pelargonium leaves, the only sets in which there is any increase at all in the respiration rates are D and E in which the respiratory  $\text{CO}_2$  was allowed to accumulate for four hours. Here, however, the increases are far too small to have any significance under the experimental conditions employed, and cannot be claimed to do more than point to a positive effect.

Sets C and F show no evidence of any effect of ionized air.

Thus there is definite evidence for a positive effect of ionized air in the case of barley seedlings but not in the case of pelargonium leaves. However, even in barley the effect is so small that it is only detectable in the very large Set A, so that it is very probable that a considerably larger effect would go unnoticed in the pelargonium sets with smaller numbers. In the case of Set F, using the indicator method, it has already been shown that an effect about four times as large as that obtained in Set A would be required for significance.

The very large increases obtained by Whimster have not been observed.



## (6) SUMMARY.

The effect of ionized air on the respiration of barley seedlings and pelargonium leaves was studied both by the continuous current method and the closed system method.

In the case of the barley seedlings a significant increase in the respiration rate of about two per cent. was found, which is smaller than that observed by Middleton (4).

With the pelargonium leaves there was no evidence of any significant effect. It is, however, probable that the methods employed were not sufficiently refined to detect an effect of the same order as that found with barley. There was no indication of any such large increase as that found by Whimster (5).

In conclusion, the author wishes to record his thanks to Professor V. H. Blackman, on whose suggestion this work was undertaken, for his unfailing interest and help; and to Dr. F. G. Gregory for much assistance with the statistical section.

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## NOTE.

### HYBRID VIGOUR IN RECIPROCAL CROSSES IN CUCURBITA PEPO.—

Ashby<sup>1</sup> has presented evidence that in maize the greater vigour of the  $F_1$  hybrid of two inbred strains is not due to a greater efficiency index than that of the parents, since it inherits, as a complete dominant, the efficiency index of the more vigorous parent, but that hybrid vigour can be accounted for by the greater size of the embryo in the hybrid grain. He shows, also, that reciprocal crosses have the same efficiency index, their difference in hybrid vigour being due to a difference in embryo size. This difference he regards as due to some process occurring between fertilization and the development of the embryo. He says, 'Hybrid vigour is nothing more than a maintenance of an initial advantage in embryo size'.

During the course of a cyto-genetical study in *Cucurbita Pepo* I have had occasion to observe the marked hybrid vigour which occurs in the  $F_1$  generations between crosses of pure lines, and some data collected from reciprocal crosses in this species may be of interest in the light of Ashby's work. The parents used in these crosses were from Professor Sinnot's inbred pure lines of *C. Pepo*. Among these lines are some with seeds which are characteristically large and are over twice the size of seeds of other lines. Since the size of the  $F_1$  seed is determined by the maternal parent it is possible, in a reciprocal cross, to get two hybrids which are genetically identical but which have widely different seed sizes. This means that they have correspondingly different embryo weights, for in the squash the greater part of the seed is embryo. Since this material lacks the complication of an endosperm, and provides such marked differences in embryo size, it seems particularly favourable for a study of this problem. Two reciprocal crosses between big-seeded and small-seeded lines were studied.

The mean weight of ten embryos was determined for each hybrid. Fifteen plants of each of the reciprocal types were grown in the field, all under the same general conditions. At intervals the number of leaves and the blade-area of each plant were recorded. After nine weeks the mature fruits were counted. At this time five plants of each series were removed from the soil and the fresh weight determined. The leaves were counted and measured. Leaf-area in this work was computed by squaring the average diameter (length plus width divided by 2), a method which, though inexact, proved suitable for comparative purposes.

The results are given in Table I. A and C represent the large-seeded parents; B and D the small-seeded ones.  $F_1$  ( $A \times B$ ) and  $F_1$  ( $B \times A$ ) are the hybrids in one reciprocal cross;  $F_1$  ( $C \times D$ ) and  $F_1$  ( $D \times C$ ) the hybrids in the other.

The figures show that the hybrids from the larger embryos are markedly larger during early development, but that the difference is much greater during the first

<sup>1</sup> Ashby, E., Studies in the Inheritance of Physiological Characters. Ann. Bot., xliv. 457, 1930. Ibid., xlv. 1007, 1932.

weeks of growth than it is later. The plants from small embryos show a tendency to catch up with those from large embryos. When the last record was made the differences in number of fruits and in weight of plant in the two types are still

TABLE I.

*Means for Measurements in the Four Hybrids of the Two Reciprocal Crosses at Four Periods during Development.*

Time in days.	Parts measured.	F <sub>1</sub> (A × B).	F <sub>1</sub> (B × A).	D/Ed.	F <sub>1</sub> (C × D).	F <sub>1</sub> (D × C).	D/Ed.
0	Weight of embryos in mg.	41 ± 2.5	18 ± 1.9	7.18	74 ± 3.2	25 ± 0.4	15.21
19	Leaf number	2.66 ± 0.1	1.66 ± 0.1	7.14	3.40 ± 0.1	2.00 ± 0.1	10.00
	Leaf area in sq. cm.	45 ± 4.9	16 ± 1.8	5.57	181 ± 6.7	45 ± 2.9	18.63 <sup>a</sup>
39	Leaf number	8.73 ± 0.21	7.33 ± 0.26	4.24	10.73 ± 0.20	8.53 ± 0.19	8.14
	Leaf area	1210 ± 101	666 ± 135	3.24	1877 ± 77	1156 ± 102	5.64
	No. of fruits	1.60 ± 0.14	1.13 ± 0.09	2.94	2.13 ± 0.07	1.73 ± 0.06	4.34
64	Weight of plant in lb.	6.75 ± 0.28	5.50 ± 0.04	4.31	5.15 ± 0.17	4.05 ± 0.30	3.12
	Leaf number	65.6 ± 0.30	65.0 ± 0.18	1.76	67.0 ± 3.90	57.1 ± 4.80	1.60
	Leaf area	27900 ± 1385	24300 ± 399	2.49	21100 ± 1560	15900 ± 1400	2.48

probably significant, but differences in leaf number and in leaf-area have ceased to be so. There appear to be two factors to consider here: (1) size of embryo, and (2) length of growth period. There is no doubt that the larger embryo gives a initial advantage which is maintained for some time. The plants in this group blossomed earlier and set fruit earlier, but at the end of nine weeks are growing more slowly, comparatively, than those from the reciprocal crosses. This indicates that the ultimate size attained is the same in the two hybrids from a reciprocal cross, but that the limit is reached first by the one that starts with the greater capital, the other attaining the limit by virtue of a longer period of growth. This fact emphasizes the importance of taking into consideration, in a study of hybrid vigour, not only initial size but duration of growth.

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